Principal Investigator: Cheek, Jeffrey M.

ABSTRACT

Environmental Tobacco Smoke and Epithelial Barrier Function

Title of Research Project (do not exceed 60 spaces)

Department of Veterinary Medicine: Anatomy, University of California at Davis

Department and Institution

The study detailed in this proposal addresses several priority issues germane to relating pulmonary health effects and exposure to tobacco smoke. Continued progress in quantitatively assessing the relative risks of acute tobacco smoke injury requires the development of approaches that are capable of not only signalling that a biologically measurable exposure has occurred, but that the exposure has exceeded the ability of the tissue or cell to repair the injury. The central goal of the proposed work is to use simple model systems (lung epithelial cell cultures) to facilitate studies of relevant dosimetry and biological mechanisms by which tobacco smoke produces epithelial cell injury in the lung. Specifically, we plan to quantitatively define the role of free radical-mediated damage, produced by exposure to environmental tobacco smoke (ETS), in the acute impairment of epithelial barrier properties *in vitro*. Our working hypothesis is that the levels of free-radical generating species present in ETS are sufficient to induce cell injury and impair the integrity and function of the pulmonary epithelial barrier, rendering the lung more susceptible to subacute and chronic disease states. Primary cultures of epithelial cells isolated from conducting airways and alveolar tissues will be used as models of free radical-mediated injury resulting from exposure to ETS *in vitro*. Changes in tissue electrophysiologic properties will provide the primary quantitative assessment of ETS effects on epithelial barrier function. The biochemical basis for the sensitivities of airway and alveolar epithelial monolayers to oxidative stress will be evaluated by assays of antioxidant enzyme activities, glutathione levels, and antioxidant nutrient status (levels of Vitamins C and E). Alterations in barrier permeability will be compared to shifts in cytoskeletal structure, intracellular calcium concentration and paracellular junctional complexes. The relationship between exposure to ETS-generated free radicals and epithelial cell injury will be characterized using histochemical approaches to identify reactive secondary oxygen species and their distribution relative to ultrastructural pathology. Methodologies for assessing the degree of ETS injury in these *in vitro* models will be evaluated for their potential in future *in vivo* applications to facilitate the defining of ETS exposure levels pertinent to health effects. These multiple strategies should improve our understanding of the nature of ETS toxicity and enable us to test protocols for alleviating acute free radical-mediated injury.

Principal Investigator: Reen Wu

ABSTRACT

Pathogenesis of Smoke-Induced Injury in Airway Epithelium

Title of Research Project (do not exceed 60 spaces)

California Primate Research Center, University of California, Davis

Department and Institution

The University of California at Davis recently acquired a state-of-the-art facility that allows us to expose small laboratory animals or cultured cells to known concentrations of chemically well-characterized environmental tobacco smoke (ETS). Only minor modifications will be needed to conduct exposure studies with mainstream tobacco smoke (MTS). The exposure facility was installed and monitored by scientists from the Analytical Chemistry Division of Oak Ridge National Laboratory, a group preeminent for their experience in tobacco smoke generation and analysis. In the PI's laboratory, a biphasic cell culture system was developed to grow and to maintain differentiated airway epithelium between the air phase and the liquid medium. This system is suitable for in vitro tobacco smoke exposure studies.

Cigarette smoking is associated with an increased risk for developing chronic bronchitis, emphysema, and lung cancer. A common feature of these diverse lung diseases is the abnormal proliferation of bronchial epithelium. The nature of this phenomenon is not known. We hypothesize that the alterations in cell proliferation may involve "autocrine and/or paracrine" regulation by growth factor(s) secreted in smoke-exposed epithelium. A preliminary study supports this hypothesis and suggests that activity like that of transforming growth factor-alpha (TGF-α) is involved in the growth regulation. To further elucidate this mechanism, we will 1) quantify dose-response relationships between concentrations of ETS or MTS, the resultant cell damage, and TGF-α gene expression; 2) elucidate the nature of the regulation of TGF-α expression by cigarette smoke in primary human and monkey tracheobronchial epithelial cultures; and 3) determine the autocrine and/or paracrine nature of the smoke-induced TGF-α in the regulation of cell growth and differentiation.

A cDNA probe and antibodies specific to TGF-α and an epidermal growth factor (EGF) receptor have been obtained. A RNA-PCR method has been developed to quantify the TGF-α message level. Nuclear run-on and inhibitor studies will be used to determine whether the regulation occurs at the transcriptional or post-transcriptional levels. In situ hybridization and immunohistochemistry will be used to identify cell type-specific gene expression. Anti-sense RNA strategies and immunological inhibition of TGF-α activity will be used to determine the nature of autocrine and/or paracrine regulation. These studies, in conjunction with a time course study, a dose-response study, and a morphological analysis will establish the role of TGF-α in tobacco smoke-induced lung diseases.

HUMAN/MONKEY

Principal Investigator: Shanna Swan, Ph.D.

ABSTRACT

Hormones and Tobacco: Joint Effects on Reproduction

Title of Research Project (do not exceed 60 spaces)

California Department of Health Services

Department and Institution

The purpose of this proposed study is to examine hormonal mechanisms for possible relationships between exposure to tobacco smoke (active and passive) and two female reproductive endpoints; subfecundability and fetal loss. Several lines of evidence suggest that smoking is anti-estrogenic, perhaps by altering steroid hormone production or metabolism, thus potentially impacting female reproductive health. These questions will be explored in the Reproductice Health Study which includes 404 married women who were noncontracepting or using barrier methods. Women provided baseline questionnaires, daily diaries and urine samples for 1-8 menstrual cycles (average 4.6). During this period, women reported 53 pregnancies, and subclinical pregnancies will be identified. Daily measurements of urinary metabolites of estrogen (E1C) and progesterone (PdG), as well as human chorionic gonadotropin (hCG) on 15 days of each cycle, are currently available on 250 women. Results from these, and the remaining assays for which funding is requested, will provide hormone profiles and data on subclinical pregnancies. We propose to reinterview women after two years to identify additional pregnancies and changes in smoking, contraception and other factors. In addition, we seek funding for three serial urinary assays for cotinine per subject which will detect changes in exposure to cigarette smoking and environmental tobacco smoke. These assays will provide an objective measure which will be compared to self reported exposures. We will examine a woman's hormone profile and tobacco exposure in relation to her reproductive experience during the 26-30 month study period. The first stage in the analysis will examine tobacco exposure in relation to ovarian function and luteal phase defects. The second stage will examine tobacco exposure in relation to fecundability and fetal loss, independent of hormonal function. In the third stage, hormonal function will be studied in relationship to fecundability and fetal loss, independent of tobacco exposure. We will then evaluate whether tobacco-related changes in hormonal parameters observed in the first stage explain altered risk of subfecundability or fetal loss in tobacco exposed women. While epidemiologic evidence suggests a relationship between (active) smoking and estrogen level, fecundability and spontaneous abortion, the strength of these associations is uncertain. Passive tobacco exposure has not been widely studied in relation to these endpoints although we found a positive association in a recent case-control study of spontaneous abortion. Abnormal steroid profiles have been linked to both infertility and habitual spontaneous abortion, but these results are based on small clinic populations. The proposed study will allow us to link laboratory data on steroid and cotinine levels to epidemiologic data on time to conception and spontaneous abortion, thus providing an opportunity to explore the hypothesis that tobacco exposure alters fecundability and risk of pregnancy loss via alteration of steroid hormones. This study will also provide distributions of urinary hormone profiles and tobacco exposures for a nonclinic, population-based sample of women which are currently unavailable.

2023470879

Principal Investigator: Wyrobek, Andrew J., Ph.D.

ABSTRACT

Tobacco smoke exposure and chromosoomally defective sperm

Title of Research Project (do not exceed 60 spaces)

Lawrence Livermore National Laboratory

Department and Institution

The objective of this research is to determine if cigarette smoking and exposure to environmetal tobacco smoke induces chromosomal damage in human sperm using a new sensitive method for detecting aneuploid sperm. Sperm carrying chromosomal aneuploidies are detected by fluorescence *in situ* hybridization with chromosome-specific repetitive-sequence DNA probes. This method was developed at our laboratory and recently validated using the human-sperm/hamster-egg cytogenetic system and human birth data. Cohybridization with multiple probes each tagged with a fluorescent dye of a different color will be used to detect several types of aneuploidies simultaneously in the same preparations: eg., 1-1, X-X, Y-Y and X-Y. This survey includes aneuploidies of autosomes as well as sex chromosomes. Fertilization with sperm carrying Y-Y and X-Y aneuploidies are predicted to yield liveborn aneuploid offspring (XYY and XXY, Klinefelter syndrome). These sperm aneuploidies, therefore, are considered biomarkers for paternally inherited genetic defects.

This research will utilize semen samples already obtained from a well characterized population of approximately 160 "normal" men. The men are partners of women enrolled in the larger Pregnancy Outcome Study conducted by the California Department of Health Services, which recruited 500 women from Kaiser Permanente Medical Center in Santa Clara, California. Husbands provided two semen samples during the menses of the first two months that their wives were enrolled. Information being obtained on this cohort (under separate funding) includes (a) tobacco smoke exposure assessment based on four different tobacco exposure indicators, (b) detailed questionnaire data regarding occupational, medical, personal, and lifestyle factors, and (c) computer-based measurements of sperm morphometry and motility performed on the same semen samples. Funding is requested to measure the fraction of aneuploid sperm in semen samples from this cohort and to perform statistical analyses to determine (a) the effects of active smoking, (b) the effects of exposure to environmental tobacco smoke and (c) the dose-response relationship between sperm aneuploidy and exposure level of active smoke. This study has an 80% power to detect a ~15% increase in mean fraction of aneuploid sperm in smokers compared with non-exposed controls.

This research will yield pivotal information on the question of whether cigarette smoking induces chromosomal damage in the sperm of active smokers and men exposed to environmental tobacco smoke.

Principal Investigator: Kass, Philip H.

ABSTRACT

Animal Models of Human Risk From Environmental Tobacco Smoke

Title of Research Project (do not exceed 60 spaces)

Department of Epidemiology and Preventive Medicine, School of Vet. Med., U.C. Davis

Department and Institution

This research will provide epidemiologic evidence concerning the association between environmental tobacco smoke (ETS) and lung cancer. We will take the novel approach of studying pet animals because household pets have greater exposures to the indoor home environment and because animals develop cancer in a much shorter time span than do humans. Dogs and cats (as well as humans) develop histologically similar forms of lung cancer, but the etiology of this cancer in non-experimental animals is unknown. Since the association between ETS and lung cancer in people remains controversial, dogs and cats represent natural *in vivo* and *in situ* models. The goals of this study are thus to:

1. Determine what association exists between lung cancer and ETS in dogs and cats.
2. Demonstrate the use of dogs and cats as sentinels of environmental health hazards in the home.

A prospective case-control study will be undertaken in California and surrounding states using a disease registry established by a veterinary pathology laboratory (California Veterinary Diagnostics, Inc.), which provides services to most veterinarians in northern California. Cases will include animals with a pathologic diagnosis of primary pulmonary carcinoma. Controls will include dogs and cats with pathologic diagnoses of splenic hematomas, reactive lymph nodes, and lipomas (conditions which are not known to be etiologically related to tobacco smoke). Owners of the pets will be interviewed and questioned about environmental tobacco smoke and other potential confounders (including other possible cancer determinants) in the home environment. These include pesticide and herbicide use, diet, and non-tobacco combustible substances released by appliances inside the home. Measures of exposures will be statistically analyzed using multivariate regression methods. If environmental tobacco smoke is found to be associated with canine or feline lung cancer, then the results add support for the causal hypothesis of ETS and human lung cancer.

2023470881

HOUSEHOLD PETS

Principal Investigator: Franklin E. Zimring

ABSTRACT

THE CHARACTER, COSTS, AND IMPACTS OF PUBLIC SMOKING PROGRAMS

Title of Research Project (do not exceed 60 spaces)

Earl Warren Legal Institute, University of California at Berkeley

Department and Institution

This project aims to provide a comprehensive statement of the costs and benefits of anti-cigarette programs. The project will assemble an annotated bibliography of assessments of smoking policies, including criminal and regulatory prohibitions, tax policies, prevention and cessation programs, passive smoking harm reduction campaigns, and smoking-related disease diagnosis and treatment programs. We will gather information on program impact on the incidence and prevalence of cigarette smoking generally, as well as program effects on specific sub-goals of cigarette policy that include child and youth prevention, smoking reduction and cessation in various target groups, passive smoking exposure, and the smoking-related mortality and morbidity. The goal is guidance on which policies might provide the least costly means to particular policy ends.

The project will survey the growing literature on smoking policies and their impacts in several nations. It will produce first an annotated bibliography on smoking policy impacts that will also cover significant studies on closely-related topics such as the impact of alcohol taxes and the effects of drug persuasion campaigns on young persons. A second publication from the project will be a monograph on the impact of smoking policy that will organize the issues and findings found in the review of the literature. This monograph will be intended for an audience of policy actors as well as scholars.

A third product of the research will be a critique of existing evaluations of smoking policy together with the identification of key issues and research strategies to study them that would be of special interest to the California Tobacco-Related Disease Research Program.

The strategic objective of this proposal is to measure our current knowledge about tobacco policy effects against the key policy issues likely to be encountered in the 1990s and beyond, to identify the significant gaps in current knowledge, and to find promising methods available to fill those gaps.

2023470882

Principal Investigator: Enoch Callaway, M.D.

ABSTRACT

The Effects of Cotinine on Human Information Processing

Title of Research Project (do not exceed 60 spaces)

Langley Porter Psychiatric Institute, University of California San Francisco

Department and Institution

The negative effects of smoking cessation on cognition have been cited as an important reason for relapse to smoking in people who are attempting to quit. Clarification of the mechanisms by which tobacco deprivation affects cognitive functioning would directly increase our understanding of the tobacco withdrawal process and lead to more effective smoking cessation treatments. Preliminary findings show that cotinine, the major metabolite of nicotine, present in the body in larger amounts and for a much longer time period than nicotine, may contribute to tobacco withdrawal symptoms by slowing information processing. Large doses of cotinine administered to subjects over several days following smoking cessation reportedly decrease tobacco withdrawal symptoms of anxiety and irritability, which may indicate relief from withdrawal of the sedative effects of cotinine, as distinct from withdrawal from nicotine itself. To our knowledge, the direct cognitive effects of such doses of cotinine have never been tested. We propose to conduct such testing on a standardized reaction time (RT) task, which has been demonstrated to be sensitive to differential effects of a variety of drugs on stimulus evaluation and response selection stages of information processing. We will use a double-blind placebo and drug pretest, posttest design and will measure RT, N100 and P300 event related potential latencies, errors, mood, blood pressure, and heart rate. We will also administer a recall memory task. Analysis will be by repeated measures ANOVAs. We will use 16 nonsmoking subjects; eight will be male and eight female, so that we may also test for gender differences in the effects of cotinine. Results will explicate cotinine's role in the tobacco withdrawal process and will provide information useful to development of cotinine as a component of smoking cessation treatment.

Principal Investigator: Witschi, Hanspeter R.

ABSTRACT

Environmental tobacco smoke and fetal growth retardation.

Title of Research Project (do not exceed 60 spaces)

Toxic Substances Research & Teaching Program, UC Davis

Department and Institution

Human epidemiological studies have shown that maternal smoking during pregnancy reduces childrens' birthweight. Whether inhalation of environmental tobacco smoke (ETS) has a similar effect is not clear. The proposed experiments are designed to test the hypothesis that ETS causes intrauterine growth retardation (IUGR) in rats. Timed-pregnant rats will be exposed to sidestream smoke, at a concentration of 1000 ug of respirable particulates (RSP)/m^3. At the end of gestation, litter size, litter weights and number of resorptions will be determined. If such an exposure causes distinct IUGR, experiments will be conducted to establish correlations between degrees of IUGR, concentrations of SS in the ambient air, and biomarkers of exposure such as plasma nicotine and cotinine. In addition we will specifically test the hypothesis that IUGR is the result of diminished placental blood flow and decreased diffusion of small nutrient molecules across the placenta because of the pharmacologic effects of nicotine. Were exposure to SS not to cause IUGR - a statement of the null hypothesis and as such of value for risk assessment purposes - we will test the secondary hypothesis that a combination of exposure to ethanol and to SS will cause fetal growth retardation in rats.

2023470584

RATS

Principal Investigator: H.P. Witschi

ABSTRACT

Environmental Tobacco Smoke and Lung Cancer

Title of Research Project (do not exceed 60 spaces)

Institute of Toxicology and Environmental Health, U.C. Davis

Department and Institution

While there is no doubt that inhalation of cigarette smoke causes lung cancer in man, it is less certain whether exposure to environmental tobacco smoke (ETS) constitutes a carcinogenic hazard. It is proposed to obtain quantitative information on the relative risk of mainstream smoke (MS) versus sidestream smoke (SS) inhalation by measuring biomarkers of effect and of exposure in hamsters. Separate groups of animals will be exposed to either SS or MS, 6 hours a day, 5 days a week, for 2, 4 or 8 weeks. Additional groups of hamsters will be allowed to recover in clean filtered air for 4 weeks following an 8 week smoke exposure. At each time point, proliferation of pulmonary cells thought to be target cells for cigarette smoke carcinogens, will be evaluated by quantitative immunohistochemistry. Data on cell proliferation will be correlated with plasma levels of nicotine and cotinine. Early molecular markers of increased cell proliferation will be sought in anatomically defined locations of the respiratory tree. These experiments will define and validate a new methodology for estimating relative risk from exposure to SS compared to MS exposure.

2023470885

HAMSTERS

Principal Investigator: Susan J. Fisher

ABSTRACT

Effect of Maternal Smoking on Human Placental Development

Title of Research Project (do not exceed 60 spaces)

Department of Stomatology, University of California, San Francisco

Department and Institution

Maternal cigarette smoking results in a number of adverse effects to the fetus, including low birth weight, prematurity, miscarriage, sudden infant death and infant mortality. We will test the hypothesis that these fetal effects are mediated, in part, by the placenta. The rationale for these studies is that the placenta exhibits a variety of abnormalities when mothers smoke during pregnancy. These are substantial in that the placenta which lies at the maternal-fetal interface, performs many important functions during pregnancy. These include uterine invasion, necessary for implantation to occur, as well as serving as an integral part of the fetal respiratory, liver, digestive and endocrine systems. Our approach will be to determine the effect of hypoxia as well as nicotine exposure on the process by which trophoblasts, the epithelial cells of the placenta that perform its various specialized functions, differentiate. Results will be correlated from both *in vitro* and *in vivo* studies which will be carried out simultaneously. Specifically we will: 1. Determine whether either hypoxia or nicotine exposure can alter human trophoblast differentiation *in vitro* using a tissue culture model of this process; and 2. Investigate whether maternal cigarette smoking alters trophoblast differentiation *in vivo* by analyzing the expression of stage-specific markers in chorionic villi and placental bed biopsies which together contain the entire trophoblast differentiation pathway. Comparison of the results from the *in vitro* and *in vivo* experiments should yield important information concerning how maternal smoking during pregnancy affects placental differentiation, and whether or not the adverse effects are related to hypoxia and/or nicotine exposure.

The results of these studies can be used to council women in much more specific terms regarding the hazards of smoking during pregnancy. For example, we may find that elements of the placental differentiation pathway responsible for establishing the utero-placental circulation are differentially and profoundly affected. This would suggest that it is extremely important for mothers not to smoke during the early stages of pregnancy when this pathway is most active. The resulting situation would be analogous to that in which many fetal systems are most sensitive to teratogenic insults during the organogensis period early in gestation. Alternatively, it is possible that we may find that placental sensitivity to maternal smoking does not change during pregnancy, suggesting that the adverse consequences to the fetus are the same no matter when the mother smokes during her pregnancy.

Principal Investigator: Derek Dunn-Rankin

ABSTRACT

Sidestream Tobacco Smoke Dispersion and Inhalability

Title of Research Project (do not exceed 60 spaces)

Department of Mechanical and Aerospace Engineering, Univ. of Calif., Irvine

Department and Institution

The proposed research studies the physical processes that control the acute exposure of non-smokers to locally high concentrations of indoor environmental tobacco smoke in situations where smoking and non-smoking areas adjoin in a single room. It is well documented that chronic exposure to environmental tobacco smoke particles has detrimental effects on human health. Before these particles can deposit in the airways, however, they must disperse from the smoke source to the nose and mouth, and then they must be inhaled or inspired. An accurate assessment of this sequence of dispersion, inhalation, and deposition, is a first step in the prediction of health risk and discomfort caused by this acute exposure. It is important to study the physical processes controlling the smoke transport, inspirability, and deposition in order to construct a model that will assign the appropriate risk to children, as well as adults, and to women as well as men. In addition to risk assessment, a model can identify potential remedies to alleviate acute exposures to sidestream smoke (e.g., erecting a partition or increasing slightly room ventilation rates). The proposed work will provide the foundation for such a model.

To construct the model, this project includes a comprehensive study of the transport of sidestream cigarette smoke from its source to human airway surfaces. The project involves both experiments and numerical simulations. The research has three components: (a) smoke transport and aging, (b) aspiration efficiency of smoke, and (c) deposition of smoke in the human airways. To examine smoke transport and aging, we will construct a chamber and a particle sizing system that will monitor the dispersion of sidestream smoke from a cigarette source to the rest of the room. In addition, we will produce a model of this dispersion for incorporation into a predictive scheme. To examine aspiration efficiency of smoke, we will modify an existing numerical model to compute the trajectories of particles in low speed flows near spherical samplers. These samplers represent the human head. We will use velocimetry measurements to validate the numerical simulations. To determine deposition of smoke, we will modify a well-developed respiratory tract deposition model to include the appropriate aspiration efficiency of smoke.

The principal product of the research is a comprehensive model that describes the relationship between environmental parameters (e.g., air circulation, partition placement), human parameters (e.g., respiration rate, body size, position relative to the smoke source), and the probable deposited dose of sidestream tobacco smoke in the respiratory tract. The project has the support of colleagues in Community and Environmental Medicine in order to ensure rigor with respect to aerosol sampling and biological aspects of the research.

2023470687

Principal Investigator: YEO, HELEN (H)

ABSTRACT

DNA Damage in Germ and Somatic Cells of Smokers

Title of Research Project (do not exceed 60 spaces)

Molecular and Cell Biology, University of California, Berkeley

Department and Institution

Tobacco smoke is known to contain high levels of oxidants. Some of these oxidants are known mutagens and are capable of damaging DNA, leading to cancer. This proposal attempts to develop a non-invasive assay to measure DNA adducts in the sperm of smokers as it would pose a risk factor for paternally induced cancers and birth defects. This finding would provide evidence that smokers are damaging their offspring. Damaged DNA is constantly repaired by DNA repair enzymes and the excised adducts are excreted in the urine where they can be assayed. Under extreme oxidative stress such as heavy smoking, we hypothesize that levels of DNA adducts in sperm and urine will be elevated.

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography with nitrogen phosphorus detection (GC-NPD) will be used as analytical tools for the detection and identification of total DNA adducts in sperm to assess the production of oxidative DNA damage *in vivo* associated with smoking. Using monoclonal antibody columns and GC-MS, the search for DNA adducts excreted in the urine of smokers will be evaluated. Furthermore, an assay using gas chromatography-mass spectrometry with selective ion monitoring (GC-MS-SIM) will be developed to measure 4-hydroxy-8-oxo-7,8-dihydroguanosine (oh^4oxo^8dG), an adduct induced by singlet oxygen, in sperm DNA and urine.

Principal Investigator: Rich, Kathryn A.

ABSTRACT

Effect of Nicotine on Pulmonary Neuroendocrine Cell Growth

Title of Research Project (do not exceed 60 spaces)

Doheny Eye Institute

Department and Institution

The children of mothers who smoke cigarettes during pregnancy have been shown to have a higher incidence of respiratory infections and and asthma during the first years of life, and a prolonged decrement in pulmonary function thereafter. Despite the well-documented epidemiological data on pulmonary impairment in the offspring of smoking mothers, the cellular processes involved in the functional alterations are unknown. In experimental animals, maternal exposure to nicotine during pregnancy has been shown to result in hyperplasia of the pulmonary neuroendocrine (PNE) cells, suggesting an alteration in their responses to growth factors. Both mammalian bombesin/gastrin-releasing peptide (GRP) and insulin-like growth factor (IGF)-I have been shown to be autocrine growth factors for human small cell lung cancer (SCLC), which is thought to derive from neoplastic transformation of PNE cells. Furthermore, SCLC secrete significant amounts of IGF-binding protein (IGFBP)-2, which may further modulate the responses of these cells to IGF-I. While a paracrine role for PNE cell-derived bombesin/GRP on the later phases of embryonic lung development has been proposed, little is known of the specific role of IGFs and IGF-binding proteins in lung cell development. Furthermore, nothing is known of the signals which cause differentiation of the PNE cell. Such signals may also be involved in the abnormal proliferation of PNE cells in various disease states.

The *hypothesis* that will be examined is that IGF-I promotes the differentiation of PNE cells in early lung development, as well as the hyperplasia of PNE cells following prenatal exposure to nicotine.

For this study, we propose to utilize an animal model system whereby nicotine is constantly infused into pregnant rats using osmotic minipumps, which results in serum nicotine levels similar to those found in human smokers. The spatial and temporal expression of the IGF-I and IGFBP-2 genes in developing rat lung, and the effects of prenatal nicotine exposure on the expression of these genes will be examined. Cell lines derived from SCLC and neuroendocrine cell tumors have been shown to exhibit receptors for nicotine, and cigarette smoke has been shown to degranulate PNE cells. Thus these cells may comprise part of the cholinergic system of the lung. However, the stage of lung development at which PNE cells acquire nicotinic receptors, and thus the period of sensitivity of the developing lung to nicotine effects *in utero*, are not known. The relative binding of radiolabelled nicotine to lung membranes at different stages of development, and whether receptor number or affinity changes in response to prenatal exposure of nicotine will be examined. *In vitro* studies with lung explants in organ culture will investigate the direct effects of nicotine and IGF-I on PNE cell differentiation, which will be assessed by the expression of GRP gene activity. Finally, the specific effects of nicotine and IGF-I on the proliferation of isolated PNE cells in primary culture will be studied. The results of these studies will provide important new information about the effects of maternal smoking on lung development and the role of nicotine and IGF-I in smoking-induced PNE cell hyperplasia.

2023470689

ABSTRACT

p53 Mutations in Sporadic and Familial Childhood Tumors

Title of Research Project (do not exceed 60 spaces)

Children's Hospital Oakland Research Institute, Children's Hospital Medical Center of Northern California

Department and Institution

Childhood tumors can be caused by tobacco mutagens, although the pathogenesis and resulting syndromes are different from those of adult tumors. The differences include a more prominent role for familial syndromes, passive smoking, and transplacental exposure. The p53 gene is an ideal tool for analyzing these phenomena, since mutations in the p53 gene reflect the effects of tobacco mutagens, are common in pediatric tumors, are implicated in familial tumors, and can be accurately characterized. The long term goal of this work is to identify the mechanisms by which tobacco causes cancer in childhood, and to develop effective clinical management programs, especially for high risk groups. In the short term, the specific objectives of this study are :

1. To determine the prevalence of p53 mutations retrospectively in childhood tumors from the Children's Hospital at Oakland (CHO) and in childhood adrenocorticocarcinomas and rhabdomyosarcomas from the Armed Forces Institute of Pathology (AFIP).
2. To further develop existing methodologies, including single-stranded conformational polymorphism analysis (SSCP) and immunohistochemistry (IHC) for rapid screening of p53 mutants from paraffin-embedded formalin-fixed tumor tissue samples.
3. To characterize the identified mutations by DNA sequencing of the p53 gene, relating the sequence changes found to characteristic changes associated with specific mutagens, as well as to the epidemiologic histories obtained, thus creating a molecular-epidemiologic profile of both familial and sporadic p53 mutations in these diagnostic groups.
4. To determine which of these patients with tumors containing p53 mutations have germ line mutations by analysis of DNA from either their somatic tissues or somatic tissue of their family members. To determine the types of p53 gene mutations encountered in families with germ line mutations in the p53 gene, and to develop the most efficient and accurate methods of diagnosis of familial p53 mutations. To investigate the role of tobacco in causing germ line p53 mutations, and the impact of tobacco in precipitating symptomatic episodes of cancer in individuals with somatic p53 mutations.
5. To immortalize lymphocytes from the peripheral blood of patients with familial syndromes for analysis of additional molecular changes or markers in the future.

2023470692

TOBACCO-RELATED DISEASE RESEARCH PROGRAM

July 1992

Grants Awarded In

Carcinogenesis, Tumor Immunology and Therapy, General Biomedical Science, and Cancer and Biomedical Science Career Development

University of California
Office of Health Affairs

Tobacco-Related Disease Research Program

July 1992

Grants Awarded In

Carcinogenesis, Tumor Immunology and Therapy, General Biomedical Science, and Cancer and Biomedical Science Career Development

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Background
Tobacco-Related Disease Research Program
University of California

In November 1988, California voters approved Proposition 99, the Tobacco Tax and Health Protection Act, which instituted a 25¢-per-pack cigarette surtax. This initiative specified that five percent of the revenue be deposited into a Research Account, to be appropriated for research on tobacco-related disease. The Tobacco-Related Disease Research Program was established by passage of SB 1613 (Chapter 1330 of the Statutes of 1989) in October 1989: "The Legislature hereby requests the University of California to establish a comprehensive grant program to support research efforts related to the prevention, causes, and treatment of tobacco-related diseases" (Section 424.20 of the Health and Safety Code).

Within the Office of the President of the University of California, specific responsibility for administering this research program was assigned to the Vice President—Health Affairs, Cornelius L. Hopper, M.D., who established the Tobacco-Related Disease Research Program (TRDRP). TRDRP has responsibility for the management of all fiscal and programmatic aspects of the grant program, while the Research Administration Office in the Office of the President manages all contractual relationships with institutions other than University of California campuses.

Direction and oversight of TRDRP are provided by a Scientific Advisory Committee which comprises representatives of various institutions with interest in the program. A list of members and the institutions they represent appears at the end of this booklet.

Research funds are available to investigators at all non-profit research institutions in California. Grant applications are peer reviewed by panels of leading investigators drawn from research institutions throughout the country. The evaluation procedure is modeled on the one used by the National Institutes of Health. In 1992, 132 reviewers evaluated applications in the following nine study sections:

Behavioral and Public Health
Carcinogenesis
Cardiovascular Disease
Epidemiology
General Biomedical Science
Pulmonary Disease
Tumor Immunology and Therapy
Cancer and Biomedical Science Career Development
Cardiovascular and Pulmonary Disease Career Development

Study sections evaluated the scientific merit of proposals and rated their responsiveness to TRDRP's current research priorities. The Scientific Advisory Committee reviewed these evaluations and recommended funding levels to the UC Vice President—Health Affairs.

In 1992 TRDRP awarded approximately $24 million to investigators at 25 institutions. The 87 grants awarded constituted more than 21 percent of applications received. From July 1990 to date, TRDRP has awarded 376 grants, for a total of approximately $118 million, to investigators at 40 California research institutions. TRDRP issues an annual report to the California Legislature which includes reports of scientific progress on active grants.

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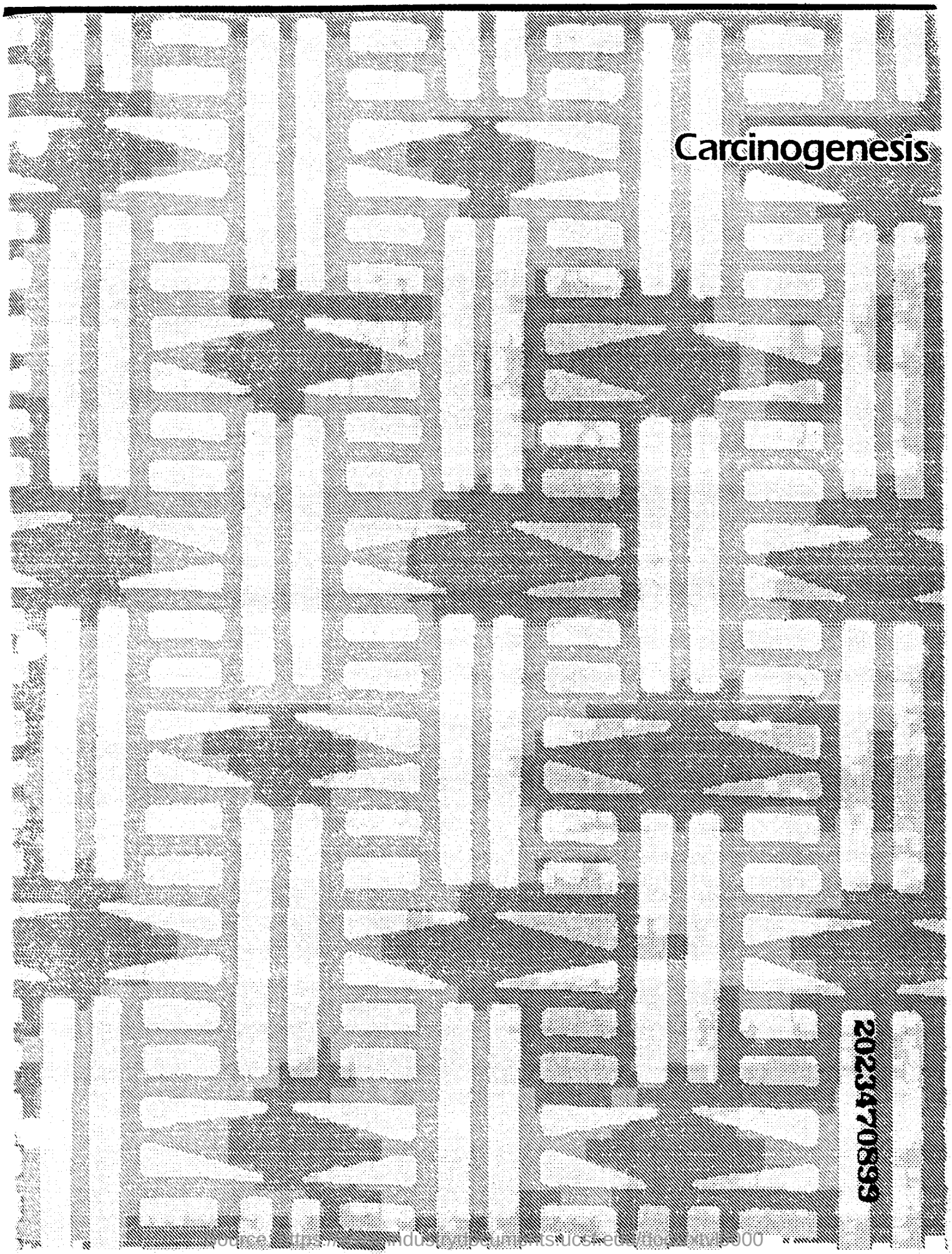

Carcinogenesis
Funded Projects

Research Awards

Campbell, Judith L.	Periodic Gene Expression in the G1 Phase of the Cell Cycle	California Institute of Technology
Derynck, Rik M.	Does TGF-b modulate Metastasis of Small Cell Lung Carcinoma	University of California, San Francisco
Donoghue, Daniel J.	Autocrine Interactions Between Bombesin and its Receptor	University of California, San Diego
Karin, Michael	Effects of Tobacco Smoke on Proto-Oncogene Activity	University of California, San Diego
Mitchison, Timothy J.	Mechanism of Mitotic Chromosome Condensation	University of California, San Francisco
Sefton, Bartholomew M.	Signal Transduction and Cell Activation	Salk Institute for Biological Studies
Waldman, Frederic M.	Tobacco Smoking & Genetic Alterations in Bladder Epithelium	University of California, San Francisco
Walter, Gernot F.	Role of Protein Phosphatase 2A in Lung Carcinoma	University of California, San Diego
Witschi, Hanspeter R.	Environmental Tobacco Smoke and Lung Cancer	University of California, Davis

Principal Investigator: Campbell, Judith L.

ABSTRACT

Periodic Gene Expression in the G1 Phase of the Cell Cycle

Title of Research Project (do not exceed 60 spaces)

Divisions of Chemistry and Biology, California Institute of Technology

Department and Institution

Tobacco, either alone, or in combination with alcohol, accounts for about 35% of the cancer cases occurring in the United States today (Doll and Peco, 1981). Most cancers arise from deregulated growth of cells which is a consequence of either activation of proto-oncogenes or inactivation of normal suppressor genes serving to constrain cell growth. Recently, the PRAD1 oncogene has been shown to be a cyclin homolog, confirming the widely held notion that an understanding of the normal cell-division-cycle with its built in regulatory mechanisms to ensure fidelity of division is there crucial to understanding the origins of cancer.

One of the master regulators of the cell cycle, the *CDC28* protein kinase was originally identified in yeast. Since then, homologs of this kinase (cyclin-dependent kinases; CDKs) have been found to be conserved through phylogeny. Together with the cyclins, the *CDC28* gene product controls entry into the S phase (where DNA duplication takes place) as well as the mitotic (nuclear division) phases of the cell cycle. Between the *CDC28* commitment step in G1 and the initiation of DNA replication in S phase, there is an induction in the message levels of 13 key replication genes at the G1/S boundary of the cell cycle.

What confers this periodicity to the DNA replication genes? As recently pointed out by several workers, all 13 of these genes have at least one copy of the sequence 5' ACGCGT 3' in their promoters. That this sequence is indeed important for cell cycle regulation has been conclusively demonstrated in this laboratory using the DNA polymerase α promoter. DNA polymerase α is a key enzyme of the replication fork involved in DNA synthesis initiation and elongation.

The elucidation of the *cis*-acting element conferring periodicity to *POL1* has set the stage for the identification and isolation of factor(s) that interact with it. Using yeast nuclear extracts fractionated by conventional and oligonucleotide affinity chromatography, a specific cell cycle element binding protein, designated MCBF, has been purified to near homogeneity. Polyclonal antisera has been raised against MCBF. A λgt11 expression library is currently being screened with the antibody to isolate the gene. Using unfractionated yeast extracts prepared from synchronized cultures, as well as from several cell division cycle mutants, an additional binding activity has been identified. Importantly, the appearance of this DNA binding complex fluctuates in the cell cycle with the same productivity as the *POL1* mRNA. Thus MCBF actually seems to be a heteromeric factor. Future experiments will be aimed at identifying the components of MCBF and determining how they sense position in the cell cycle. Isolating the MCBF genes by such an approach and creating mutants in each will help us elucidate downstream events such as initiation of DNA replication. Upstream events, such as whether cdc28 protein kinase (the evolutionarily conserved cell cycle master regulator) activates the *trans*-acting factors will also be studied. These series of experiments will further our understanding of the normal cascade of cell cycle events. This in turn is crucial for understanding how regulated checkpoints of the cell cycle are bypassed when the cell becomes oncogenic, proliferating uncontrollably.

A second major and concurrent goal, since we have now established an assay for induction of MCBF in G1, will be to use this assay to test the effect of components of tobacco smoke on the induction of MCBF and of *POL1* mRNA.

Principal Investigator: Derynck, Rik

ABSTRACT

Does TGF-β modulate metastasis of small cell lung carcinoma?

Title of Research Project (do not exceed 60 spaces)

Growth & Development, University of California San Francisco

Department and Institution

Tobacco smoking contributes considerably to the development of lung cancers, among which small lung cell carcinoma is a predominant type. It is not the formation of the primary tumor but the dissemination of the tumor due to metastasis is responsible for the severity and the high mortality of many cancers. Tumor cells frequently synthesize higher levels of transforming growth factor-β, suggesting that TGF-β may exert autocrine effects that play a role or even provide an advantage to tumor cell behavior. TGF-β has a strong ability to regulate the expression of genes that are important in interactions of the cells with the extracellular matrix. **As a result of these activities, TGF-β can modulate in an autocrine way the interaction between cell and matrix and increase matrix deposition and decrease its degradation. Thus, it is likely that endogenous TGF-β expression level in tumor cells is an important determinant of the invasive and metastatic behavior of tumor cells, and that alterations in endogenous TGF-β expression level will modulate, presumably decrease, the metastatic behavior.**

We propose **to study the influence of the endogenous TGF-β expression level on invasiveness and spontaneous metastasis** of a small cell lung carcinoma cell line. Using specific TGF-β expression vectors for overexpression of TGF-β or abolition of TGF-β expression (by overexpressing dominant negative TGF-β mutants) we will generate transfected cell lines, all derived from the same parental small cell lung carcinoma line, that either overexpress high levels of natural "latent" TGF-β or activated TGF-β, or have undergone virtual abolition of endogenous TGF-β expression. These cell lines will be evaluated for several parameters *in vitro* and *in vivo* that are important for or indicative of invasiveness and metastasis. *In vitro*, we will evaluate cell proliferation and glycolysis, adhesiveness and integrin expression, expression of extracellular matrix-degrading proteases and invasion in an *in vitro* assay. *In vivo*, we will evaluate subcutaneous tumor formation and spontaneous metastasis in nude mice. Several aspects of tumor formation will be evaluated: incidence of tumor formation, kinetics of tumor growth, histological appearance and invasiveness. The spontaneous metastasis of these tumor cells will be determined by a combination of macroscopic and histological examination and a molecular evaluation. With respect to the latter aspect, the extent of metastasis will be measured by determining the levels of chloramphenicol acetyltransferase (CAT), an enzyme specifically only expressed by the tumor cells due to an initial transfection with a CAT expression vector. The latter method of measuring the level of metastasis should provide a great level of accuracy and an extreme sensitivity, which cannot be obtained using conventional methods.

This study should allow an evaluation of the effects of alterations of endogenous TGF-β expression and the resulting autocrine/partacrine effects on the invasiveness and metastasis of small cell lung carcinoma cells. Insight in the role of autocrine/paracrine effects of endogenous TGF-β synthesis in invasiveness and metastasis is not only important for our understanding of metastasis of the many tobacco-related or unrelated cancers, but could also open up TGF-β based therapeutic modalities to interfere with metastasis.

2023470902

Donoghue, Daniel J.

SECTION III. ABSTRACT

AUTOCRINE INTERACTIONS BETWEEN BOMBESIN AND ITS RECEPTOR

Department of Chemistry, University of California San Diego

This proposal aims to examine the autocrine interactions between bombesin-related growth factors and the bombesin receptor. Many studies have reported the overproduction of bombesin-related peptides by small cell lung carinoma (SCLC) cells, which also exhibit cell surface bombesin receptors, establishing the existence of an autocrine loop between this growth factor and its receptor. However, these studies have not investigated whether intracellular activation of the bombesin receptor by bombesin-related peptides may occur in autocrine transformed cells. Thus, the use of anti-bombesin antibodies for the treatment of SCLC poses a major unanswered problem concerning the intracellular activation of bombesin receptors. The potential treatment of SCLC by immunotherapies directed against autocrine growth factors may have a serious limitation if bombesin-related peptides can interact intracellularly with the bombesin receptor. It is the goal of this proposal to experimentally address this issue.

In a first set of experiments, a cell line will be constructed which allows *inducible* expression of mammalian bombesin, or gastrin-releasing peptide (GRP). The autocrine interactions between mammalian bombesin and its receptor will be examined following induced synthesis of the growth factor. Induced tyrosine phosphorylation of cellular proteins will be monitored. Mitogenic stimulation will be determined, as will the transcriptional induction of nuclear protooncogenes such as c-*fos*. Inhibitors of protein transport in the secretory pathway will be exploited in an effort to ascertain whether interactions between the growth factor and its receptor can occur intracellularly, or are confined to the cell surface.

Additional experiments will construct mutants of mammalian bombesin that allow intracellular retention of a *membrane-anchored* form. Mutations will be introduced into the DNA sequence encoding mammalian bombesin so as to alter the known sites of posttranslational processing. These mutations will include: a) mutation of the processing site used to generate the N-terminus of mature bombesin; b) replacement of the signal sequence of mammalian bombesin with the signal/anchor sequence of neuraminidase to allow membrane-anchoring; c) replacement of the signal sequence with mutant forms of the neuraminidase signal/anchor which will lead to membrane-anchoring as well as retention in the endoplasmic reticulum (ER) or Golgi. These studies will allow examination of autocrine interactions between membrane-anchored forms of bombesin with its receptor.

Principal Investigator: Karin, Michael

ABSTRACT

Effects of Tobacco Smoke on Proto-Oncogene Activity

Title of Research Project (do not exceed 60 spaces)

Department of Pharmacology, University of California, San Diego

Department and Institution

Lung cancer accounts for almost one third of all cancer deaths in the USA. Approximately 90% of the deaths from lung cancer can be traced directly to smoking. Although a complete ban on cigarette smoking is the most effective way to reduce the incidence of lung cancer, such a drastic countermeasure at the time being is unrealistic. In the meantime, it is essential to understand in better detail the mechanisms contributing to lung carcinogenesis. Such an understanding would lead to development of diagnostic assays for early detection of lung cancer and possibly even to an effective therapy. Cigarette smoke contains a number of proven carcinogens and tumor promoters. While the tumor initiation step is more or less understood, the mechanism of tumor promotion requires further investigation. Our previous work indicates that the AP-1 transcriptional activating complex, composed of the cJun and cFos oncoproteins, is a major mediator of the effects of tumor promoters on gene expression. Furthermore, agents that inhibit AP-1 activity, such as retinoic acid, are potent anti-tumor promoters and the consumption of green and yellow vegetables rich in retinoic acid precursors is associated with lower lung cancer risks. Since various findings point to the *c-jun* protooncogene as a major mediator of tumor promotion, we propose to investigate the mechanisms by which tumor promoters and carcinogens present in cigarette smoke affect its expression and AP-1 activity. In addition, we will determine how activated *ras* genes, which have been implicated in lung cancer, cooperate with *c-jun* during the carcinogenic process. Since the basic mechanisms of growth control are identical in all diploid non-transformed cells we will use for these studies primary cultures of rat embryo fibroblasts. These cells will be exposed to various agents present in cigarette smoke whose effects on *jun* and *fos* gene expression and AP-1 activity will be assessed by blot hybridization and mobility shift assays. We will examine how those agents found to increase *c-jun* expression affect the activity of the *c-jun* promoter. We will compare the mechanism by which these agents induce *c-jun* transcription to the one used by phorbol ester tumor promoters. A variety of transfection and biochemical experiments will be used to determine how expression of an activated Ras protein affects the activity of the cJun protein. We will pay special attention to the affects of Ras and other oncoproteins involved in lung carcinogenesis on cJun phosphorylation. We will purify and characterize an oncogene activated protein kinase responsible for the stimulation of cJun activity. These studies should provide us with detailed information regarding the mechanism of tumor promotion by substances present in cigarette smoke. Better understanding of the biochemical events underlying the development of lung cancer is an important prerequisite for the rational design of drugs that will interrupt and possibly reverse this oncogenic process.

2023470904

Principal Investigator: Mitchison, Timothy J.

ABSTRACT

Mechanism of mitotic chromosome condensation

Title of Research Project (do not exceed 60 spaces)

Dept. Pharmacology University of California, San Francisco

Department and Institution

Prior to dividing in two at mitosis cells must condense their DNA into mitotic chromosomes. The condensation process is an important basic step in the G2 phase of the cell-cycle, but its mechanism is unknown. Chromosome condensation in extracts of Xenopus (toad) eggs is blocked by an important lung-cancer drug, etoposide [Adachi et al 1991, Hirano and Mitchison 1991]. This drug binds to the enzyme topoisomerase-II, but the mechanism by which it kills cancer cells is unknown. Since the drug kills cells in G2, it may do so by blocking chromosome condensation. We propose to study the molecular mechanism of condensation in Xenopus extracts. We hope to determine whether condensation is an active or passive process, and which chromosomal proteins drive condensation. We also hope to determine the role of topoisomerase-II in condensation, and how etoposide blocks the process. Our results will provide fundamental information about the cell-biology of cancer cells. They will also tell us how an important anti-lung cancer drug acts, and provide a system for testing other drugs that may block chromosome condensation.

2023470905

Principal Investigator: Sefton, Bartholomew M.

ABSTRACT

Signal Transduction and Cell Activation

Title of Research Project (do not exceed 60 spaces)

Molecular Biology and Virology Laboratory, The Salk Institute

Department and Institution

This project is basic research designed to understand the role of tyrosine protein phosphorylation in the activation of B lymphocytes by antigen. Its relevance to the goals of the Tobacco-related disease research program is that it will provide insights into the regulation of cell proliferation, which is crucial to an understanding of cancer, and into the activation of hematopoietic cells, which is of importance to an understanding of the role of neutrophils in smoke-induced lung damage. The binding of antigen or anti-immunoglobulin antibody to immunoglobulin M or D on the surface of mature B cells induces the rapid phosphorylation of approximately 10 proteins on tyrosine. Surface immunoglobulin therefore must be coupled in some manner to one or more tyrosine protein kinases or phosphatases. Because an inhibitor of tyrosine phosphorylation renders B cells unresponsive to stimulation through the antigen receptor, tyrosine phosphorylation is apparently important in B cell activation. B cells that do not express the tyrosine protein phosphatase CD45 are unresponsive to the cross-linking of surface immunoglobulin. This insensitivity could be due to increased inhibitory phosphorylation of a tyrosine protein kinase. We will therefore study the phosphorylation of the several *src*-family tyrosine protein kinases found associated with the antigen receptor complex. Additionally, we will ask whether the insensitivity of CD45-negative B cells is reversed by introduction into them of a constitutively-activated version of a src-family kinase. We will also study the interaction of the src-family kinases with the antigen receptor complex. We will identify the domain of the src kinases responsible for their interaction by introduction into B cells of mutated src kinases. Finally, we will exploit newly-isolated monoclonal antibodies to two novel tyrosine phosphorylated proteins in activated B cells. The 95K and 140K proteins that these antibodies recognize will be characterized and their genes cloned. These studies have the potential to reveal the identities of tyrosine protein kinases and substrates that are important in the regulation of the proliferation of normal B cells and may play a role in the unregulated growth of malignant B cells such as those transformed by Abelson murine leukemia virus.

Principal Investigator: Waldman, Frederic M

ABSTRACT

TOBACCO SMOKING & GENETIC ALTERATIONS IN BLADDER EPITHELIUM

Title of REsearch Project (do not exceed 60 spaces)

Division of Molecular Cytometry - Laboratory Medicine - University of California, San Francisco

Department and Institution

Nationwide, more than 50,000 people will be diagnosed with urinary bladder cancer in 1992, and more than 10,000 will die from this disease. Tobacco smoking increases the risk of bladder cancer 2-4 times, and it is estimated that more than 50% of bladder cancers are caused by tobacco use. It is thought that smoking increases the risk of bladder cancer by formation of DNA adducts, leading to tumorigenic genetic aberrations in uroepithelial cells. In order to investigate the association of bladder cancer and smoking, we propose to study the levels of chromosomal aberrations in interphase nuclei from bladder epithelial biopsies obtained from smokers and nonsmokers. Normal bladder epithelium from smokers and nonsmokers will come from autopsy specimens and from patients undergoing urological surgery for nonmalignant diseases (50-100 per year). Morphologically normal appearing bladder biopsies from patients having concurrent bladder tumors (25-50 per year) will also be analyzed for numerical and structural aberrations. Smoking histories of all patients will be determined by review of charts (autopsy patients) or by direct questioning by physicians. Numerical chromosomal abnormalities of chromosomes 7, 9, 11, 17, X, and Y will be characterized on a cell by cell basis using fluorescence in situ hybridization (FISH) with chromosome-specific repetitive DNA probes. Structural aberrations will be investigated using FISH with cosmid DNA probes specific for erbB2, p53, and EGF-R, and 9q. S phase fraction of all biopsies will be determined by *in vitro* 5-bromodeoxyuridine incorporation as an indicator of cell proliferation. Data will be analyzed to test whether there is a smoking-related increase in background genetic aberrations.

Principal Investigator: Gernot Walter, Ph.D.

ABSTRACT

Role of Protein Phosphatase 2A in Lung Carcinoma

Title of Research Project (do not exceed 60 spaces)

Department of Pathology, University of California, San Diego

Department and Institution

Protein phosphatase 2A (PP2A) is the major soluble form of protein serine/threonine phosphatase in most tissues and cells. Studies with okadaic acid, tumor antigens, and mitosis promoting factor indicate that PP2A plays an inhibitory role in cell proliferation. Increasing the intracellular level of PP2A should therefore reduce the rate of proliferation. The goal of this proposal is to introduce PP2A into tumor cells under the control of an inducible promoter, and to test if induction of PP2A results in growth inhibition. The specific aims are: (1) To add antigenic determinants (tags) to the carboxy and/or amino termini of the catalytic and regulatory subunits (C and A subunits, respectively) of PP2A in order to distinguish "exogenous" from "endogenous" cellular subunits. (2) To construct inducible vectors for the controlled expression of the "tagged" PP2A subunits by using the MMTV promoter and lac operator-repressor systems. (3) To introduce the inducible subunit expression vectors into transformed rat fibroblasts and human lung carcinoma cells in order to study the effects of subunit over-expression on cell proliferation, signal transduction pathways, and phosphorylation states of specific oncogene and tumor suppressor gene products.

Principal Investigator: H.P. Witschi

ABSTRACT

Environmental Tobacco Smoke and Lung Cancer

Title of Research Project (do not exceed 60 spaces)

Institute of Toxicology and Environmental Health, U.C. Davis

Department and Institution

While there is no doubt that inhalation of cigarette smoke causes lung cancer in man, it is less certain whether exposure to environmental tobacco smoke (ETS) constitutes a carcinogenic hazard. It is proposed to obtain quantitative information on the relative risk of mainstream smoke (MS) versus sidestream smoke (SS) inhalation by measuring biomarkers of effect and of exposure in hamsters. Separate groups of animals will be exposed to either SS or MS, 6 hours a day, 5 days a week, for 2, 4 or 8 weeks. Additional groups of hamsters will be allowed to recover in clean filtered air for 4 weeks following an 8 week smoke exposure. At each time point, proliferation of pulmonary cells thought to be target cells for cigarette smoke carcinogens, will be evaluated by quantitative immunohistochemistry. Data on cell proliferation will be correlated with plasma levels of nicotine and cotinine. Early molecular markers of increased cell proliferation will be sought in anatomically defined locations of the respiratory tree. These experiments will define and validate a new methodology for estimating relative risk from exposure to SS compared to MS exposure.

2023470909

TOBACCO-RELATED DISEASE RESEARCH PROGRAM

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Tumor Immunology and Therapy

Tumor Immunology & Therapy
Funded Projects

Research Awards

Cheresh, David A.	Role of aV Integrins in Malignancy of Tobacco/Induced Tumors	The Scripps Research Institute
Epstein, Alan L.	Radioimmunotherapy of Lung Cancer	University of Southern California
Langmuir, Virginia K.	Radioimmunotherapy & Hypoxic Cytotoxin in Squamous Cancer	SRI International
Moran, Richard G.	Response of Respiratory Cancers to Novel Antiofolates	University of Southern California
Nobori, Tsutomu	Diagnosis and Treatment of MTAse-Deficient Lung Cancers	University of California, San Diego
Shastri, Nilabh	T-Cell Stimulating Antigen Genes in Tumor Cells	University of California, Berkeley

IDEA Awards

Kleinfeld, Alan M.	Serum Free Fatty Acid Levels in Patients with Malignancies	Medical Biology Institute
Schnitzer, Jan E.	Tumor-Specific Vascular Proteins in Lung Cancer	University of California, San Diego

Principal Investigator: DAVID A. CHERESH, PH.D.

ABSTRACT

ROLE OF αV INTEGRINS IN MALIGNANCY OF TOBACCO-INDUCED TUMORS

Title of Research Project (do not exceed 60 spaces)

DEPARTMENT OF IMMUNOLOGY, THE SCRIPPS RESEARCH INSTITUTE

Department and Institution

Human lung and pancreatic carcinoma in many cases may be the direct result of tobacco use. These tumors, in particular, are among the most deadly known to man with mortality rates in the range of 90-95% thought to be the result of their propensity to metastasize to local, regional or distant sites. Their metastatic behavior is potentiated by the capacity of individual tumor cells to attach, migrate and invade host extracellular matrices and basement membranes. Considerable progress has been made toward understanding the molecular basis of these biological processes. To this end a family of cell adhesion receptors termed integrins has been defined that potentiate these cellular events. Among the major integrins on lung and pancreatic carcinoma are the αv integrins which serve as their primary or exclusive vitronectin and fibronectin receptors. The αv integrin subunit is unique in that it associates with a wide variety of β subunits each of which has unique adhesive and biological properties. Thus, the objective of this proposal will be to determine the role of αV integrins in the malignant phenotype of human lung and pancreatic carcinoma. The specific aims to accomplish these goals are as follows:

1. To determine whether vitronectin and/or fibronectin expressed in cryostat sections of human or mouse lymphnode and liver can serve as an adhesive ligand(s) for metastatic lung or pancreatic carcinoma cells that were previously selected for metastasis to lymphnodes, lungs or liver in vivo. This will include a determination as to whether one or multiple αv integrin mediate this adhesion event and if any of these adhesion receptors are expressed to a variable extent on the metastatic cells.

2. To investigate whether the structurally unique cytoplasmic tail of the β5 subunit is responsible for the failure of integrin αvβ5 on lung or pancreatic carcinoma cells to establish focal contacts and associate with the actin cytoskeleton in response to vitronectin as is normally observed with αvβ3 or αvβ1. Studies will be designed to elucidate the structural basis of αv integrins association with the actin cytoskeleton in the carcinoma cell.

3. To examine the role of αv integrins in the migration of carcinoma cells and to compare migratory and chemotactic responses of FG and UCLA-P3 parental carcinoma cells to their metastatic counter parts. This involves the determination as to whether the cytoplasmic domain of individual β chains play a role in this process by examining the migratory properties of these carcinoma cells transfected with various chimeric β subunit.

Principal Investigator: Epstein, Alan L.

ABSTRACT

Radioimmunotherapy of Lung Cancer

Title of Research Project (do not exceed 60 spaces)

Pathology Department, USC School of Medicine

Department and Institution

The primary objective of this study is to determine the clinical effectiveness of radiolabeled monoclonal antibodies (MAbs) to unique intracellular antigens as a means of selectively targeting human lung cancers which contain abnormally permeable, degenerating cells. Previous studies have shown that a high proportion of malignant tumors undergo degeneration and cell death with the formation of necrosis. In addition, the inadequate blood supply and impaired phagocytic responses within the tumor favor the accumulation of degenerating cells adjacent to viable areas. In contrast, normal tissues have a relatively low rate of cell death and are characterized by a rapid and orderly removal of necrotic elements. Based upon these observations, we hypothesized that MAbs to abundant intracellular antigens which are structural components of the cell and are therefore retained by degenerating cells, may be used to target a wide range of human malignancies. To test this hypothesis, several major studies have been performed in our laboratory using monoclonal antibody TNT-1 (IgG2a) directed against nuclear histone H1. The first demonstrated conclusively that TNT-1 can image human tumor transplants in nude mice with no apparent uptake by normal tissues. The second showed that when labeled with I-131, TNT-1 can be used as an effective treatment modality in a human cervical carcinoma-nude mouse model. A major advantage of this approach is that with the use of multiple doses of I-131 labeled TNT-1, an enlarging area of degeneration and necrosis is formed within the tumor thus providing an ever expanding target for TNT-1 therapy (gangrene-like effect). Autoradiographic studies demonstrated that TNT-1 can penetrate and bind in a selective manner to both early and advanced necrotic regions in tumors. Finally, initial clinical imaging studies in man using I-131 labeled TNT-1 F(ab')2 fragments showed selective uptake in 5/7 tumors including a sarcoma, and pancreatic, breast, colon, and cervical carcinomas and provided verification of this experimental approach in man.

In this proposal, we intend to extend these studies by developing a genetically engineered TNT-1 MAb which can be used safely in patients without evoking an immunological response. A chimeric MAb containing murine variable regions from TNT-1 will be combined with human γ_1 constant region sequences will be constructed in a mammalian expression system. In addition, using the same TNT-1 variable region sequences, single chain antibodies will be constructed in a bacterial expression system. The biological activity of the newly generated chimeric and single chain TNT-1 Mabs will be compared with their murine TNT-1 counterpart with respect to stability, avidity of binding, imaging, biodistribution, and pharmacokinetics in lung carinoma-bearing nude mice. Both subcutaneous and intrabronchial lung carcinoma tumor models will be used to generate experimental data applicable to the patient. It is anticipated that the chimeric TNT-1 will have therapeutic use while the fast clearing single chain variant will be used for patient imaging studies. The data produced by these studies will enable the preparation of a federally approved IND for the use of radiolabeled chimeric and single chain TNT-1 MAbs for the treatment of lung cancers and related malignancies in terminally ill patients.

ABSTRACT

Radioimmunotherapy and Hypoxic Cytotoxin in Squamous Cancer

Title of Research Project (do not exceed 60 spaces)

Cell and Molecular Biology Laboratory, SRI International

Department and Institution

Radioimmunotherapy (RIT) has shown some promising results in clinical trials, however it is unlikely that macroscopic tumors will be cured by RIT alone. Because hypoxic cells are not well targeted by radiolabeled antibody, combining RIT with hypoxic cytotoxins should be efficacious. Human squamous cell carcinomas of the head and neck have been shown to have hypoxic regions in many patients. This tumor type is therefore an appropriate one in which to study this combined modality therapy.

The Specific Aims of this project are twofold: **1. To determine the efficacy of RIT plus benzotriazine di-N-oxide hypoxic cytotoxins in an in vivo tumor model using squamous cell carcinoma of the head and neck; and 2. To evaluate the mechanisms of interaction between these two therapeutic modalities by studying drug distribution and the occurrence of tumor reoxygenation and rehypoxiation during treatment.**

Hypothesis 1: Combining RIT and an hypoxic cytotoxin in SCC of the head and neck significantly enhances tumor cell killing and slows tumor regrowth in nude mice when compared to either modality given alone. Our preliminary studies using ^{131}I-labeled antibody plus SR 4233, an hypoxic cytotoxin developed here at SRI, in a human colon cancer model suggest that this hypothesis is true. However the cell line used was much more radiosensitive than most human solid tumors. For this reason, it would be useful to study a tumor that is more radioresistant. HN-5, a human squamous cell carcinoma of the tongue is such a tumor and it will be grown as a xenograft in nude mice. Treatment will be radiolabeled antibody (using ^{125}I labeled with an antibody to the epidermal growth factor receptor which is internalized and ^{131}I labeled with an antibody to a surface antigen that is not internalized) and hypoxic cytotoxin (benzotriazine di-N-oxides developed here at SRI), given alone and in combination. Endpoints will be clonogenic assay and tumor regrowth delay.

Hypothesis 2: The efficacy of this combined therapy is due in part to targeting of different cell populations but is significantly enhanced by reoxygenation and reestablishment of the hypoxic fraction during treatment. Targeting of the radiolabeled antibody and the drug will be evaluated using autoradiography. Reoxygenation/rehypoxiation studies will be done by measuring the hypoxic fraction at varying times after the start of treatment. As this requires a large number of mice and gives no indication of where the hypoxic cells are, a new immunohistochemical technique will also be used to estimate hypoxic fraction. If these mechanisms do not fully account for the efficacy of combined therapy, future studies could evaluate the roles of DNA damage and repair.

Principal Investigator: Richard G. Moran, Ph.D

ABSTRACT

RESPONSE OF RESPIRATORY CANCERS TO NOVEL ANTIFOLATES

Title of Research Project (do not exceed 60 spaces)

Biochemistry and the Norris Comprehensive Cancer Center, University of Southern California

Department and Institution

Chronic inhalation of tobacco smoke is the major risk factor for the development of carcinomas of the lung and, together with alcohol intake, is a major factor predisposing to tumors of the head and neck. There is sufficient evidence for the activity of folate antimetabolites and 5-fluorouracil in the treatment of some of these diseases to suggest that the activity of inhibitors of folate-dependent pathways, particularly thymidylate synthase could prove quite active againsst these diseases and should be optimized on a cell type by cell type basis. If there were better understanding of the reasons for sensitivity, refractoriness, and the development of resistance on a biochemical, cellular and molecular basis, these goals would be attainable. Recently, new classes of folate antimetabolites have been developed that have no activity against the classical target for antifolates, dihydrofolate reductase, but rather have thymidylate synthase and the folate-dependent enzymes of *de novo* purine synthesis as sites of action. The two prototypes of these classes of drugs, ICI-D1694 and DDATHF, respectively, are currently completing Phase 1 clinical trials and should soon be ready for disease-specific trials.

We propose to study the susceptibility of human lung carcinomas and head and neck carcinomas to the potent new classes of folate antimetabolites that have thymidylate synthase and *de novo* purine synthesis as targets. These studies will have three components: the first will characterize the responsiveness of these cell types to in vitro cytotoxicity and biochemical studies, the second will determine the interaction of these compounds with the rate limiting enzyme determining sensitivity to such drugs in respiratory tumors and whether there are multiple species of this enzyme, and the third will study the processes that determine recovery from and resistance to these drugs.

2023470917

Principal Investigator: Tsutomu Nobori, M.D.

ABSTRACT

DIAGNOSIS AND TREATMENT OF MTAse-DEFICIENT LUNG CANCERS

Title of Research Project (do not exceed 60 spaces)

Medicine/University of California, San Diego

Department and Institution

In the United States, lung cancer is the leading cause of death in men and the second leading cause in women. Smoking is responsible for 85% of lung cancer deaths among men and 75% among women. In California, it is estimated in 1991 that the total new cases of lung cancer will be 16,100 and that total deaths from lung cancer will be 13,900. Because symptoms often don't appear until the disease is in advanced stages, early detection of lung cancer is very difficult. The 5-year relative survival rate is only 13% in all patients, regardless of stage at diagnosis. Therefore, any gains made in prevention or new methods of early detection or treating this disease, even if they affect only a small percentage of the total cases, would have a large impact in terms of total lives saved.

The broad, long-term objectives of this research proposal are to determine the prevalence and clinical characteristics of methylthioadenosine phosphorylase (MTAse) deficiency in human non-small cell lung cancer (NSCLC) and to develop and apply new methods for the specific treatment of MTAse-negative NSCLC.

In order to detect MTAse-negative primary NSCLC, we will develop simple and accurate screening methods that will include the quantification of protein levels of MTAse in lung cancer tissue specimens, using the immunoblotting procedure, and the detection of the enzyme deficiency in tissue sections and sputum specimens by immunostaining. To develop new methods for the selective treatment of MTAse-negative NSCLC, we will use dideazatetrahydrofolate, a potent inhibitor of *de novo* purine synthesis, and purified or recombinant L-methioninase, a methionine-degrading enzyme, in combination with MTA in both *in vitro* systems and in animal models.

In mammalian cells, methylthioadenosine (MTA) is produced during the synthesis of the polyamines. MTA does not accumulate in normal tissues but is cleaved rapidly to adenine and 5'-methylthioribose 1-phosphate (MTR-1-P) by MTAse. The adenine and MTR-1-P are recycled to purine nucleotides and methionine, respectively. Since all normal cells or tissues are known to contain MTAse, MTAse deficiency in NSCLC will enable us to develop tumor-specific chemotherapy, in which MTAse-negative cancer cells will be selectively killed with drugs causing the depletion of purine nucleotides or methionine, under conditions where MTAse-positive normal lung tissues can be rescued by giving MTA as the sources of purine nucleotides or methionine.

Principal Investigator: Shastri, Nilabh

ABSTRACT

T-Cell Stimulating Antigen Genes in Tumor Cells

Title of Research Project (do not exceed 60 spaces)

Molecular and Cell Biology, Regents of the University of California Berkeley

Department and Institution

This project aims to identify antigen genes expressed in the Lewis lung carcinoma (3LL) cells. These genes will be isolated using a novel and unique expression cloning strategy for T-cell defined antigens we have established in this laboratory. LacZ inducible, cytotoxic T-cell clones (CTL.Z) will be generated which recognize endogenous peptides bound by either the K^b or the D^b class I MHC molecules on surface by 3LL cells. These CTL.Z cells will be used as single cell indicators to isolate the corresponding antigenic peptide coding gene(s) in a 3LL cDNA library by expression cloning. For this purpose we have constructed, (a) a novel derivative of BW5147 fusion partner which allows construction of lacZ inducible, T-cell hybrids specific for any given antigen, and (b) stable K^b or D^b expressing COS transfectants which allow high efficiency of gene transfer and express the peptide/MHC complex on their surface. With the isolated antigen coding cDNA clones, we will identify the precise peptides responsible for the peptide/MHC complex recognized by the CTL.Z cells, as well as, analyze the expression of the antigen encoding mRNA in a variety of normal and tumor tissues. Knowledge of which CTL stimulating genes are expressed in 3LL cells are likely to provide, (a) an explanation for intriguing observations relating the metastatic potential of 3LL cells to surface K^b/D^b MHC expression, (b) the foundation for future studies for development of therapeutic approaches to inhibit tumor growth, as well as, for understanding the transformed state itself.

Principal Investigator: Kleinfeld, Alan M.

ABSTRACT

Serum Free Fatty Acid Levels in Patients with Malignancies

Title of Research Project (do not exceed 60 spaces)

Division of Membrane Biology, Medical Biology Institute

Department and Institution

Immunosuppression is a common correlate of advanced cancer. The mechanisms for this decrease in immune function are likely multifactorial. Evidence for a direct role of cis unsaturated free fatty acids (FFA) in tumor related immunosuppression has been found in *in vitro* and *in vivo* studies. *in vitro* studies have demonstrated that relatively low levels of cis FFA inhibit cytotoxic T lymphocyte (CTL) mediated killing of tumor cells. This effect may have important consequences for physiological tumorigenesis since recent studies show that tumor cells, in contrast to most normal cells, constitutively release FFA and that upon CTL attack this level of release increases more than tenfold. The absolute levels of cis FFA released in this process could easily exceed the levels found to be inhibitory. Moreover, studies in humans and animals with fully developed malignancies show serum elevations of total fatty acid (FA) and this serum has been shown to be immunosuppressive. These observations suggest that elaboration of FFA by tumor cells may represent an auto-protective mechanism intrinsic to the ability of early tumor clones to escape clearance by cell mediated cytotoxicity.

The objective of the proposed study is to extend the very limited clinical data regarding FA levels in patients with tobacco related malignancies. Extensive studies of this sort have not been done previously because a unifying hypothesis for such measurements was lacking and because no method existed for the direct measurement of FFA levels. Determination of total FA, which can be measured and used to estimate FFA levels, is not sufficiently accurate nor practical on a large scale. We have recently developed a fluorescent probe that allows direct, rapid, and accurate measurement of [FFA], thereby eliminating the technical barriers to such a study. Thus, in the proposed study we will carry out measurements to establish whether elevated FFA levels are a signature of tobacco related cancers and possibly of cancer, in general.

If FFA levels are found to be elevated then the FFA probe technology could become an important adjunct to cancer diagnosis and treatment. Sera of cancer patients could easily be studied and correlated with known prognostic features such as stage of disease and therapeutic intervention. Elevated FFA levels would provide strong support for a role of FFA in tumorigenesis and would suggest new strategies for treatment of cancer.

Principal Investigator: Schnitzer, J. E.

ABSTRACT

nor-specific Vascular Proteins of Lung Cancer

Title of Research Project (do not exceed 60 spaces)

Dept. of Medicine; UCSD School of Medicine; Div. Cellular & Molecular Med.
Department and Institution

Tobacco is the most important cause of cancer in the United States, playing a role in about one-third of all cancer cases. Lung cancer is the number one source of all cancers and smoking is its primary cause. In 1990, over 150,000 new cases were estimated to have occurred in the United States of America from lung cancer alone. In this study, we propose to examine lung cancer from a different perspective by focusing at the molecular level on the lung tumor vasculature. Vascular development clearly plays a central role in tumor growth and metastasis. Morphologically, the vasculature of tumors is distinctly different from that of normal tissue. This project will investigate the molecular differences between the vasculature of normal and neoplastic tissue of the lung in order to create specific diagnostic and therapeutic probes. Proteins associated with the luminal surface of the pulmonary endothelium of normal and neoplastic tissue will be identified and compared by utilizing a novel membrane-isolation scheme that directly isolates luminal endothelial membranes from tissue in situ. In this technique, the vascular wall is selectively coated by perfusion through the pulmonary artery. After homogenization of the lung tissue, selective sedimentation centrifugation is used to isolate endothelial membranes by taking advantage of the greater density of the coated membrane sheets than any other component of the homogenate. This technique has been optimized in normal rat lung tissue and shows excellent purification of endothelial membranes as assessed both by electron microscopy and by immunoblotting for endothelial markers such angotensin converting enzyme. We now wish to apply this technique to isolating luminal endothelial membranes from lung tumors so that proteins specific for this vasculature can be identified and purified. We will use this approach on various cancer models in rats and mice. The proteins will be analyzed by one and two-dimensional gel electrophoresis. Antibodies will be made to tumor-specific proteins of interest in order to develop probes specific for the vasculature of the tumor. These specific antibodies can then be used as a diagnostic tool for detecting tumor growth in the experimental animal models and to immunolocalize protein expression in various normal and neoplastic tissues. With antibodies specific for the lung tumor vasculature, therapeutic targeting of the tumor vasculature may also be possible. Because it is clear that the tumor vasculature and its full development are critical for tumor growth, metastasis, maintenance and probably survival, specific ablation of the tumor blood supply should cause significant tumor regression.

2023470921

TOBACCO-RELATED DISEASE RESEARCH PROGRAM

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General Biomedical Science

General Biomedical Science
Funded Projects

Research Awards

Comings, David E.	The Dopamine D2 Receptor in Nicotine Addiction	City of Hope National Medical Center
Dunn-Rankin, Derek	Sidestream Tobacco Smoke Dispersion and Inhalability	University of California, Irvine
Fisher, Susan J.	Effect of Maternal Smoking on Human Placental Development	University of California, San Francisco
Keyser, Kent T.	Acetylcholine Receptors in the Developing Nervous System	University of California, San Diego
Kraemer, Fredric B.	Effects of Nicotine on Weight	Stanford University
Ochoa, Enrique L.	Desensitization of Cholinergic Mechanisms & Nicotine Addictn	University of California, Davis
Witschi, Hanspeter R.	Environmental Tobacco Smoke and Fetal Growth Retardation	University of California, Davis

IDEA Awards

Edmond, John	Environmental Tobacco Smoke & the Brain Growth Spurt	University of California, Los Angeles

2023470925

Principal Investigator: Comings, David E.

ABSTRACT

THE DOPAMINE D2 RECEPTOR IN NICOTINE ADDICTION

Title of Research Project (do not exceed 60 spaces)

Department of Medical Genetics, City of Hope Medical Center

Department and Institution

While tobacco smoking is the leading cause of lung cancer, addiction to nicotine is the leading cause of smoking and thus the real cause of lung cancer. Understanding and preventing this addiction may be the single most effective way of preventing lung cancer. The leading theory of addictive behavior centers around stimulation of the dopaminergic reward pathways in the brain. A genetic defect in the function of this pathway may explain why some individuals are so susceptible to addictive behaviors while others are not. The dopamine D2 receptor gene has been cloned and shown to be associated with a Taq I restriction endonuclease fragment length polymorphism (RFLP). Our studies show this allele is strongly associated with polysubstance abuse. Additional alleles have recently been described allowing more precise haplotyping of the DRD2 gene. Preliminary results indicate some of these haplotypes show even greater association with polysubstance abuse than the D2A1 allele. We propose to examine the frequency of the D2A1 allele and different DRD2 haplotypes in individuals who smoke heavily compared to controls. This research may allow the identification of individuals who are especially predisposed to addictive behaviors and lead to methods of preventing nicotine addiction and thus lung cancer.

Principal Investigator: Derek Dunn-Rankin

ABSTRACT

Sidestream Tobacco Smoke Dispersion and Inhalability

Title of Research Project (do not exceed 60 spaces)

Department of Mechanical and Aerospace Engineering, Univ. of Calif., Irvine

Department and Institution

The proposed research studies the physical processes that control the acute exposure of non-smokers to locally high concentrations of indoor environmental tobacco smoke in situations where smoking and non-smoking areas adjoin in a single room. It is well documented that chronic exposure to environmental tobacco smoke particles has detrimental effects on human health. Before these particles can deposit in the airways, however, they must disperse from the smoke source to the nose and mouth, and then they must be inhaled or inspired. An accurate assessment of this sequence of dispersion, inhalation, and deposition, is a first step in the prediction of health risk and discomfort caused by this acute exposure. It is important to study the physical processes controlling the smoke transport, inspirability, and deposition in order to construct a model that will assign the appropriate risk to children, as well as adults, and to women as well as men. In addition to risk assessment, a model can identify potential remedies to alleviate acute exposures to sidestream smoke (e.g., erecting a partition or increasing slightly room ventilation rates). The proposed work will provide the foundation for such a model.

To construct the model, this project includes a comprehensive study of the transport of sidestream cigarette smoke from its source to human airway surfaces. The project involves both experiments and numerical simulations. The research has three components: (a) smoke transport and aging, (b) aspiration efficiency of smoke, and (c) deposition of smoke in the human airways. To examine smoke transport and aging, we will construct a chamber and a particle sizing system that will monitor the dispersion of sidestream smoke from a cigarette source to the rest of the room. In addition, we will produce a model of this dispersion for incorporation into a predictive scheme. To examine aspiration efficiency of smoke, we will modify an existing numerical model to compute the trajectories of particles in low speed flows near spherical samplers. These samplers represent the human head. We will use velocimetry measurements to validate the numerical simulations. To determine deposition of smoke, we will modify a well-developed respiratory tract deposition model to include the appropriate aspiration efficiency of smoke.

The principal product of the research is a comprehensive model that describes the relationship between environmental parameters (e.g., air circulation, partition placement), human parameters (e.g., respiration rate, body size, position relative to the smoke source), and the probable deposited dose of sidestream tobacco smoke in the respiratory tract. The project has the support of colleagues in Community and Environmental Medicine in order to ensure rigor with respect to aerosol sampling and biological aspects of the research.

2023470927

Principal Investigator: Susan J. Fisher

ABSTRACT

Effect of Maternal Smoking on Human Placental Development

Title of Research Project (do not exceed 60 spaces)

Department of Stomatology, University of California, San Francisco

Department and Institution

Maternal cigarette smoking results in a number of adverse effects to the fetus, including low birth weight, prematurity, miscarriage, sudden infant death and infant mortality. We will test the hypothesis that these fetal effects are mediated, in part, by the placenta. The rationale for these studies is that the placenta exhibits a variety of abnormalities when mothers smoke during pregnancy. These are substantial in that the placenta which lies at the maternal-fetal interface, performs many important functions during pregnancy. These include uterine invasion, necessary for implantation to occur, as well as serving as an integral part of the fetal respiratory, liver, digestive and endocrine systems. Our approach will be to determine the effect of hypoxia as well as nicotine exposure on the process by which trophoblasts, the epithelial cells of the placenta that perform its various specialized functions, differentiate. Results will be correlated from both *in vitro* and *in vivo* studies which will be carried out simultaneously. Specifically we will: 1. Determine whether either hypoxia or nicotine exposure can alter human trophoblast differentiation *in vitro* using a tissue culture model of this process; and 2. Investigate whether maternal cigarette smoking alters trophoblast differentiation *in vivo* by analyzing the expression of stage-specific markers in chorionic villi and placental bed biopsies which together contain the entire trophoblast differentiation pathway. Comparison of the results from the *in vitro* and *in vivo* experiments should yield important information concerning how maternal smoking during pregnancy affects placental differentiation, and whether or not the adverse effects are related to hypoxia and/or nicotine exposure.

The results of these studies can be used to council women in much more specific terms regarding the hazards of smoking during pregnancy. For example, we may find that elements of the placental differentiation pathway responsible for establishing the utero-placental circulation are differentially and profoundly affected. This would suggest that it is extremely important for mothers not to smoke during the early stages of pregnancy when this pathway is most active. The resulting situation would be analogous to that in which many fetal systems are most sensitive to teratogenic insults during the organogensis period early in gestation. Alternatively, it is possible that we may find that placental sensitivity to maternal smoking does not change during pregnancy, suggesting that the adverse consequences to the fetus are the same no matter when the mother smokes during her pregnancy.

2023420928

Principal Investigator: Kent T. Keyser

ABSTRACT

Acetylcholine Receptors in the Developing Nervous System

Title of Research Project (do not exceed 60 spaces)

Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CA 92093

Department and Institution

Acetylcholine is used as a transmitter in many portions of the vertebrate nervous system and much information is available concerning the neurons that release it. However, the *cholinoceptive* neurons have been more to difficult to identify and for this reason less information is available concerning the postsynaptic elements of cholinergic circuits. During the past several years various groups have purified nicotinic acetylcholine receptors (nAChRs) from the nervous system and have raised antibodies against them. These antibodies have proven to be extremely useful in studies of nAChRs in the central and peripheral nervous system. However, recent studies suggest that the cells that express the known nAChRs may not be the only cholinoceptive neurons in the nervous system. Specifically, α subunits of α-bungarotoxin sensitive acetylcholine receptors (αBgtAChRs) have been isolated from brain and have been shown to form channels that respond to ACh and nicotine when expressed in *Xenopus* oocytes. This suggests that the αBgtAChRs represent a new class of acetylcholine receptors. The proposed research program will investigate the expression of nAChRs and αBgtAChRs in the brain and retina during embryogenesis. Previous studies have shown that these receptors are highly sensitive to cholinergic agents and, unlike other classes of receptors, increase in number in the CNS in response to chronic exposure to nicotine. Thus, knowledge of the time of onset and sequence of expression of nAChRs and αBgtAChRs in specific areas of the nervous system of embryonic animals is essential in order to evaluate the possible effects of pre-natal exposure to nicotine. The proposed research program is comprised of three sections.

1) Immune precipitation experiments that rely upon monoclonal antibodies directed against nAChR and αBgtAChR subunits will be used to measure: a) when during development the production of the individual nAChR and αBgtAChR subtypes begins and b) relative amounts of the different receptor subtypes in brain and retina throughout the various stages of embryogenesis.

2) *In situ* hybridization studies using riboprobes will be carried out in embryonic brain and retina to determine: a) the time of onset of expression of the different nAChR and αBgtAChR subunit mRNAs and b) the distribution of receptor subunit mRNAs throughout the brain and retina at various developmental times.

3) In order to determine what areas of the nervous system are producing immunologically detectable amounts of the receptor subunits, the same antibodies used in the immune precipitation studies will be used to map where in the developing brain and retina the different nAChR and αBgtAChR subtypes are produced. In addition, these studies will determine if the expression pattern is constant or if it changes as development proceeds.

Principal Investigator: Kraemer, Fredric B.

ABSTRACT

Effects of Nicotine on Weight

Title of Research Project (do not exceed 60 spaces)

Department of Medicine, Stanford University

Department and Institution

Smoking and cessation of smoking are associated with significant changes in weight. Individuals who begin to smoke usually lose weight and individuals who stop smoking generally experience a significant weight gain. Indeed, the fear of gaining weight is often seen as a major obstacle for individuals who attempt to stop smoking. While there is some controversy whether the changes in weight that occur with smoking are associated with alterations in food consumption or in energy expenditure, it is likely that behavioral events are not exclusively responsible and that physiological pathways influenced by nicotine contribute to these changes in weight since the modifications in weight observed in humans who smoke can be duplicated in experimental animals by the infusion of nicotine under conditions where no alterations in food consumption and physical activity occur. Surprisingly little information is available concerning the metabolic perturbations that lead to the changes in weight with smoking and cessation. The overall goal of this proposal is to understand the cellular mechanisms responsible for the sustained decrease in weight induced by nicotine and the increase in weight that accompanies nicotine withdrawal by directing studies at understanding the effects of nicotine on adipose tissue metabolism. The hypotheses to be tested are: 1) Nicotine, either directly or indirectly, stimulates lipolysis and reesterification of stored triglycerides within adipocytes, resulting in a futile cycle and thereby an increased energy utilization; and 2) The weight loss associated with smoking is due to alterations in the regulation of lipoprotein lipase induced, either directly or indirectly, by nicotine. In order to test these hypotheses, rats will be studied following the acute and chronic administration of nicotine, as well as following the withdrawal of chronic nicotine infusion. The first hypothesis will be tested by measuring rates of lipolysis and reesterification in isolated adipocytes. In addition, the sensitivity of adipocytes isolated from nicotine treated animals to lipolytic and antilipolytic agents will be determined. If altered as expected, the individual steps in the pathway of lipolysis that are affected by nicotine will be assessed by measuring the number of adrenergic and adenosine receptors, the activity of adenylate cyclase, and the activity, mass and mRNA expression of hormone sensitive lipase. The second hypothesis will be tested by measuring the activity, mRNA levels, mass, and catalytic activity of lipoprotein lipase in adipose tissue from rats following the acute and chronic administration of nicotine, as well as following the withdrawal of chronic nicotine infusion. Additionally, changes in the response of adipose tissue lipoprotein lipase from nicotine treated animals to hormonal regulators will be examined. These studies should provide evidence for the mechanistic events underlying nicotine-induced alterations in weight. Understanding the mechanisms responsible for changes in weight among smokers has significant implications for developing regimens that promote smoking cessation while preventing excessive weight gain and should be viewed as an attempt to understand the basis of one aspect of the psychological dependence which develops with smoking and, as such, an important part of the overall scheme in comprehending the many facets of nicotine addiction.

2023470930

Principal Investigator: Enrique L.M. Ochoa, M.D., Ph.D.

ABSTRACT

DESENSITIZATION OF CHOLINERGIC MECHANISMS AND NICOTINE ADDICTION

Title of Research Project (do not exceed 60 spaces)

Pediatrics:Neonatology, University of California, Davis

Department and Institution

Chronic administration of nicotine up-regulates the number of adult and fetal brain [^{3}H] nicotine binding sites, a paradoxical phenomenon correlated with inactivation of function (i.e., desensitization) of nicotinic receptors at the molecular level, and with behavioral symptoms ranging from tolerance, dependence, and withdrawal to nicotine in the adult, to attention deficit disorders and other cognitive syndromes of childhood. The long-term goal of this project is to provide a detailed description of nicotine-induced inactivation (i.e., desensitization) of nicotinic cholinergic mechanisms at the pre- and post-synaptic levels. Desensitization may have different characteristics in different subtypes of neuronal nAchRs, and related nicotinic cholinergic pre-synaptic mechanisms may have particular desensitization profiles. It is hypothesized that acute inactivation of central nicotinic cholinergic neurotransmission is the basis for long-term modifications in receptor number and operation, which ultimately determine tolerance to the drug. Molecular information about the individual components of central cholinergic synapses, including a characterization of their desensitization properties, is still lacking.

This research proposal will combine biochemical and molecular biological techniques currently used for characterizing peripheral cholinergic synapses to gain insight into the function of the related central cholinergic mechanisms. In one approach, synaptosomes will be prepared from rat brain striatum and hippocampus and the nicotine concentrations required to activate and desensitize transmitter release will be explored. The role of key factors such as Ca^{2+} will receive especial attention. Desensitization of release will be correlated with the phosphorylation state of specific synaptic vesicle proteins (e.g.: synapsins I and II). The other type of approach includes the expression of functional neuronal nAChrs by injecting *Xenopus laevis* oocytes with the messenger RNAs prepared from specific cDNAs corresponding to each of the receptor subunits. Electrophysiological techniques will be used to monitor the currents associated with receptor activation and desensitization. Different pairwise combinations of α and β neuronal subunits will be scrutinized with regards to their nicotine-induced desensitizaton profile.

It is anticipated that the combined molecular biological and neurochemical approaches described in this proposal will provide new basic information for understanding neural mechanisms expressed at behavioral levels. Furthermore, a detailed characterization of nicotine actions on cholinergic neurons could elucidate the basic mechanisms underlying other severe drug addictions, since nicotine specifically acts on the mesolimbic system, a neuroanatomical pathway involved in repetitive self-administration of many commonly abused drugs.

Principal Investigator: Witschi, Hanspeter R. [redacted]

ABSTRACT

Environmental tobacco smoke and fetal growth retardation.

Title of Research Project (do not exceed 60 spaces)

Toxic Substances Research & Teaching Program, UC Davis

Department and Institution

Human epidemiological studies have shown that maternal smoking during pregnancy reduces childrens' birthweight. Whether inhalation of environmental tobacco smoke (ETS) has a similar effect is not clear. The proposed experiments are designed to test the hypothesis that ETS causes intrauterine growth retardation (IUGR) in rats. Timed-pregnant rats will be exposed to sidestream smoke, at a concentration of 1000 ug of respirable particulates (RSP)/m^3. At the end of gestation, litter size, litter weights and number of resorptions will be determined. If such an exposure causes distinct IUGR, experiments will be conducted to establish correlations between degrees of IUGR, concentrations of SS in the ambient air, and biomarkers of exposure such as plasma nicotine and cotinine. In addition we will specifically test the hypothesis that IUGR is the result of diminished placental blood flow and decreased diffusion of small nutrient molecules across the placenta because of the pharmacologic effects of nicotine. Were exposure to SS not to cause IUGR - a statement of the null hypothesis and as such of value for risk assessment purposes - we will test the secondary hypothesis that a combination of exposure to ethanol and to SS will cause fetal growth retardation in rats.

2023470932

DOOR

Principal Investigator: John Edmond, Ph.D.

ABSTRACT

ENVIRONMENTAL TOBACCO SMOKE AND THE BRAIN GROWTH SPURT.
Title of Research Project (do not exceed 60 spaces)

Mental Retardation Research Center, UCLA School of Medicine.
Department and Institution

Two specific aims are presented to begin to address the hypothesis that environmental tobacco smoke disrupts the massive growth spurt in developing brain. This proposal originates from the observation that children born to individuals who actively smoke during their pregnancy show a reduced birth weight. It is proposed this outcome is the consequence of a failure to maintain an adequate energy status to meet the energy dependent needs for rapid growth and development. The rat pup, artificially reared on milk substitutes, is to serve as the model system to test the proposals. This model is favored because the critical growth spurt in rat brain occurs postnatally in the milk feeding period between the 8th and 14th day after birth. The crux of our hypothesis is based on our very latest findings that the two major lipids for membranes in brain, cholesterol and palmitate, are synthesized *de novo* entirely by the brain to meet its needs. Thus, adequate and timely provision of these lipids is subverted when there is inadequate energy provision to support the dynamic processes of neuronal cell development and lipogenesis for membrane synthesis. Since the brain growth spurt and onset of myelinogenesis are developmentally programmed events, their delay or omission will result in permanent abnormalities of function. The direct effect of exposure to tobacco smoke is to be studied on the artificially reared rat in the period 5 to 16 days of age which encompasses the period of the major brain growth spurt. One specific aim deals with establishing conditions of exposure to smoke which will result in blood cotinine concentrations of about 300ng/ml as representative of the conditions observed for active smoking, whereas blood cotinine concentrations of 3 and 30 ng/ml are projected to represent two conditions of passive exposure. The second specific aim is directed at a close examination of the cellular (neuronal) organization/structure of brain and its regions by a morphological analyses involving the application of immunohistochemical/histological methods,

The proposal relates to conditions for the human because, like the rat, the human fetus/baby experiences a brain growth spurt coincident with the onset of myelination which occurs over the third trimester of pregnancy through the first four months after birth. Since brain growth is active in the human newborn at a period which parallels that in rat pup brain, our model system is expected to discover, by a direct approach, whether our hypothesis is valid. Should the general growth reductions which have been reported for the human infant exposed to environmental tobacco smoke extend to the critical events of the brain growth spurt then the mechanisms by which tobacco smoke perturbs these processes can be identified.

TOBACCO-RELATED DISEASE RESEARCH PROGRAM

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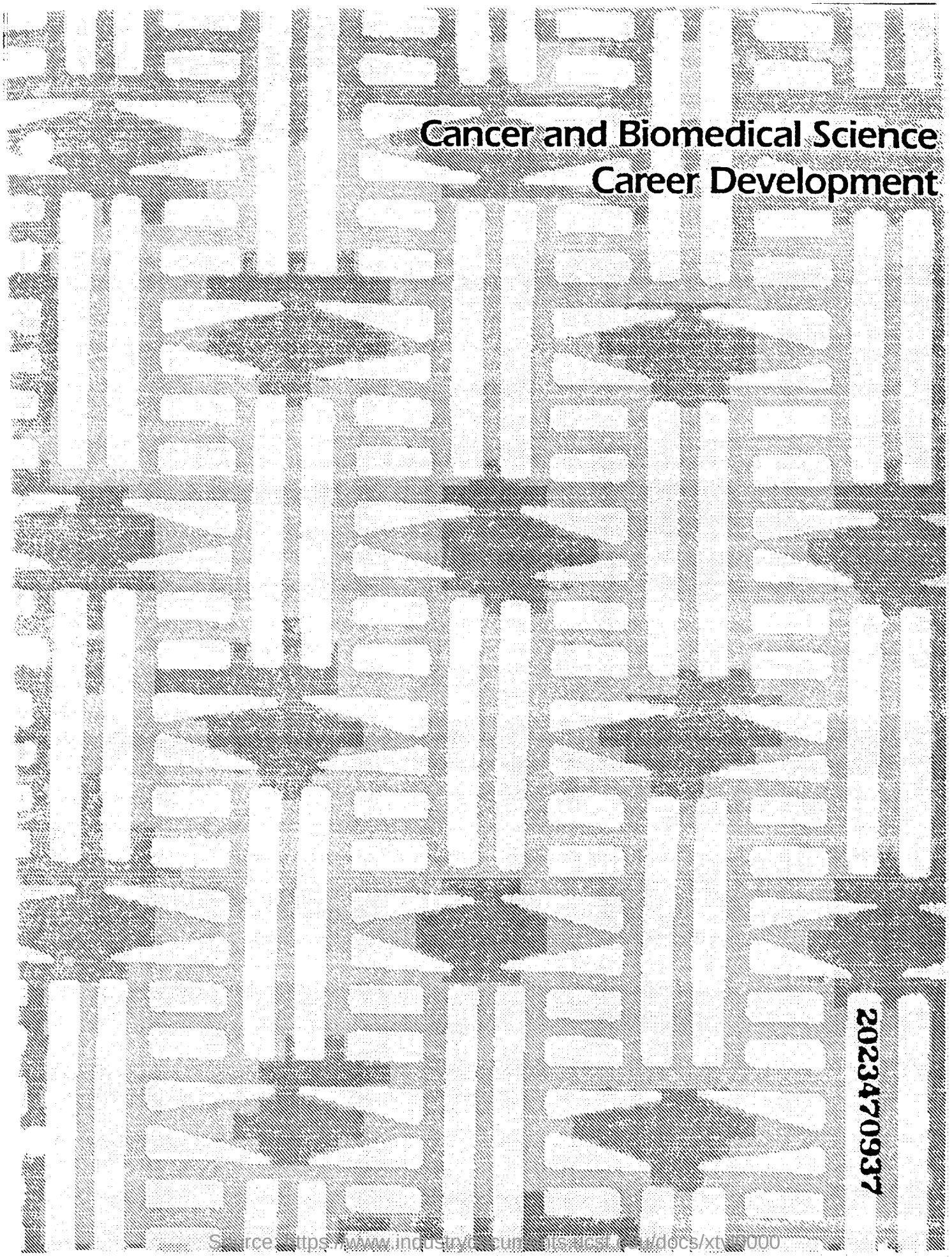
Cancer and Biomedical Science Career Development



Cancer and Biomedical Science and Career Development
Funded Projects

New Investigator Awards

Bodner, Sara M.	p53 Mutations in Sporadic & Familial Childhood Tumors	Children's Hospital Med. Ctr. of No. CA
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Rich, Kathryn A.	Effects of Nicotine on Pulmonary Neuroendocrine Cell Growth	Doheny Eye Institute
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M[illegible] Churl K.	Molecular Mechanisms of Brain Nicotinic Receptor Inactivatn	California Institute of Technology
Minden, Audrey G.	Regulation of the NGFI-A Protein by Phosphorylation	University of California, San Diego
Sheridan, Philip L.	Biochemical and Functional Characterization of HIP-116	Salk Institute for Biological Studies
Shum, Lillian	Signal Transduction by TGF-d Precursor During Transformation	University of California, San Francisco
Verma, Rati	Cell Cycle Regulation of Proliferation Genes	California Institute of Technology
Yeo, Helen C.	DNA Damage in Germ & Somatic Cells of Smokers	University of California, Berkeley
Yoon, Hye-Joo	The Mechanics and Regulation of Centromere Function	University of California, Santa Barbara

Principal Investigator: Sara M. Bodner, M.D.

ABSTRACT

p53 Mutations in Sporadic and Familial Childhood Tumors

Title of Research Project (do not exceed 60 spaces)

Children's Hospital Oakland Research Institute, Children's Hospital Medical Center of Northern California

Department and Institution

Childhood tumors can be caused by tobacco mutagens, although the pathogenesis and resulting syndromes are different from those of adult tumors. The differences include a more prominent role for familial syndromes, passive smoking, and transplacental exposure. The p53 gene is an ideal tool for analyzing these phenomena, since mutations in the p53 gene reflect the effects of tobacco mutagens, are common in pediatric tumors, are implicated in familial tumors, and can be accurately characterized. The long term goal of this work is to identify the mechanisms by which tobacco causes cancer in childhood, and to develop effective clinical management programs, especially for high risk groups. In the short term, the specific objectives of this study are :

1. To determine the prevalence of p53 mutations retrospectively in childhood tumors from the Children's Hospital at Oakland (CHO) and in childhood adrenocorticocarcinomas and rhabdomyosarcomas from the Armed Forces Institute of Pathology (AFIP).
2. To further develop existing methodologies, including single-stranded conformational polymorphism analysis (SSCP) and immunohistochemistry (IHC) for rapid screening of p53 mutants from paraffin-embedded formalin-fixed tumor tissue samples.
3. To characterize the identified mutations by DNA sequencing of the p53 gene, relating the sequence changes found to characteristic changes associated with specific mutagens, as well as to the epidemiologic histories obtained, thus creating a molecular-epidemiologic profile of both familial and sporadic p53 mutations in these diagnostic groups.
4. To determine which of these patients with tumors containing p53 mutations have germ line mutations by analysis of DNA from either their somatic tissues or somatic tissue of their family members. To determine the types of p53 gene mutations encountered in families with germ line mutations in the p53 gene, and to develop the most efficient and accurate methods of diagnosis of familial p53 mutations. To investigate the role of tobacco in causing germ line p53 mutations, and the impact of tobacco in precipitating symptomatic episodes of cancer in individuals with somatic p53 mutations.
5. To immortalize lymphocytes from the peripheral blood of patients with familial syndromes for analysis of additional molecular changes or markers in the future.

Principal Investigator: Chun, Jerold J.M.

ABSTRACT

Nicotinic Receptor Biology in CNS Neurons: An In Vitro Model

Title of Research Project (do not exceed 60 spaces)

Dept. of Pharmacology, University of California, San Diego

Department and Institution

Tobacco-related diseases stem from the continued use of tobacco. This use is stimulated by the addictive constituent in tobacco, nicotine. An understanding of the molecular mechanisms of nicotine addiction could lead to effective therapeutic strategies for disrupting or preventing nicotine addiction. While the molecular mechanisms responsible for nicotine addiction are unknown, they must involve neurons of the central nervous system (CNS) that recognize nicotine and respond to it. Recent molecular evidence indicates that the neural gene families with homology to the α and β subunits of the muscle nicotinic cholinergic receptor are receptors for nicotine on CNS neurons. Nevertheless, it has thus far been virtually impossible to study these subunits in a neuronal CNS system because of the complexity and heterogeneity of the CNS. Moreover, the molecular changes occurring in CNS neurons after continued nicotine exposure - changes that must play a role in addiction - have not been experimentally approachable.

This proposal addresses the interaction of CNS neurons with nicotine by taking an approach of proven use in the study of the complex immune and muscle systems: using cell lines. A new protocol based on retroviral targeting of neurons is employed for making clonal, stable neuron-like lines. These lines will be used to study CNS nicotinic receptors in neurons. Standard tissue culture, electrophysiologic and molecular biologic techniques will be used. Cell lines will be produced, screened for nicotinic receptor presence (or absence: the null phenotype) and categorized according to their receptor subunit phenotype. Each category will be analyzed further by electrophysiology of endogenously or exogenously (transfected) expressed receptors and compared to previous oocyte studies. Appropriate cell lines will further be used to identify and study the cis- and trans-acting elements. Lastly, appropriate cell lines will be used to identify induced genes following transient and chronic repeated exposure to nicotine by using subtractive hybridization techniques. Genes identified through this procedure will be further studied by in situ hybridization and molecular/physiologic approaches in appropriate categories of derived cell lines.

Principal Investigator: Rich, Kathryn A.

[redacted]

ABSTRACT

Effect of Nicotine on Pulmonary Neuroendocrine Cell Growth

Title of Research Project (do not exceed 60 spaces)

Doheny Eye Institute

Department and Institution

The children of mothers who smoke cigarettes during pregnancy have been shown to have a higher incidence of respiratory infections and and asthma during the first years of life, and a prolonged decrement in pulmonary function thereafter. Despite the well-documented epidemiological data on pulmonary impairment in the offspring of smoking mothers, the cellular processes involved in the functional alterations are unknown. In experimental animals, maternal exposure to nicotine during pregnancy has been shown to result in hyperplasia of the pulmonary neuroendocrine (PNE) cells, suggesting an alteration in their responses to growth factors. Both mammalian bombesin/gastrin-releasing peptide (GRP) and insulin-like growth factor (IGF)-I have been shown to be autocrine growth factors for human small cell lung cancer (SCLC), which is thought to derive from neoplastic transformation of PNE cells. Furthermore, SCLC secrete significant amounts of IGF-binding protein (IGFBP)-2, which may further modulate the responses of these cells to IGF-I. While a paracrine role for PNE cell-derived bombesin/GRP on the later phases of embryonic lung development has been proposed, little is known of the specific role of IGFs and IGF-binding proteins in lung cell development. Furthermore, nothing is known of the signals which cause differentiation of the PNE cell. Such signals may also be involved in the abnormal proliferation of PNE cells in various disease states.

The *hypothesis* that will be examined is that IGF-I promotes the differentiation of PNE cells in early lung development, as well as the hyperplasia of PNE cells following prenatal exposure to nicotine.

For this study, we propose to utilize an animal model system whereby nicotine is constantly infused into pregnant rats using osmotic minipumps, which results in serum nicotine levels similar to those found in human smokers. The spatial and temporal expression of the IGF-I and IGFBP-2 genes in developing rat lung, and the effects of prenatal nicotine exposure on the expression of these genes will be examined. Cell lines derived from SCLC and neuroendocrine cell tumors have been shown to exhibit receptors for nicotine, and cigarette smoke has been shown to degranulate PNE cells. Thus these cells may comprise part of the cholinergic system of the lung. However, the stage of lung development at which PNE cells acquire nicotinic receptors, and thus the period of sensitivity of the developing lung to nicotine effects *in utero*, are not known. The relative binding of radiolabelled nicotine to lung membranes at different stages of development, and whether receptor number or affinity changes in response to prenatal exposure of nicotine will be examined. *In vitro* studies with lung explants in organ culture will investigate the direct effects of nicotine and IGF-I on PNE cell differentiation, which will be assessed by the expression of GRP gene activity. Finally, the specific effects of nicotine and IGF-I on the proliferation of isolated PNE cells in primary culture will be studied. The results of these studies will provide important new information about the effects of maternal smoking on lung development and the role of nicotine and IGF-I in smoking-induced PNE cell hyperplasia.

2023470942

Principal Investigator: Ruoss, Stephen J.

ABSTRACT

Role of Mast Cell Tryptase in Tobacco-Induced Carcinogenesis

Title of Research Project (do not exceed 60 spaces)

Cardiovascular Research Institute and Department of Medicine, UC-San Francisco

Department and Institution

Tobacco smoke-induced airway epithelial carcinogenesis is a complex, multi-step process, influenced by a variety of factors. Emerging evidence suggests an important role for the proliferative activity of cells in the evolution of a malignant clone following carcinogen exposure. Mast cells, normal residents of human airway subepithelium, are increased in number in cigarette smokers and actively release mediators, including a mast cell-specific protease, tryptase, into the airway epithelium and lumen. Recent research has established potent growth factor activity of this specific protease for fibroblasts and epithelial cells in culture. Given the increased release of active tryptase in the airways of smokers, its subepithelial location, and its established mitogenic activity in appropriate target cells, it is now proposed that tryptase may play an important role in the evolution of tobacco related lung cancer. This mast cell-specific mitogen could participate in *in vivo* carcinogenesis by increasing the mitotic rate of epithelial cells exposed to tobacco-derived carcinogens, or by acting as a local proliferative agonist for an established malignant cell clone. These questions will be examined through the use of established cultured epithelial cell lines and animal models of tumor formation. Epithelial cells from human and other mammalian airway sources will be exposed *in vitro* to tobacco-derived carcinogens and purified mast cell tryptase. Multiple parameters will be followed, including growth rates, responses to additional mitogens, and induction of gene transcription associated with neoplastic progression. Evidence of neoplastic transformation following tryptase and tobacco-derived carcinogen exposure will be measured by the ability of the cells to grow under anchorage-independent conditions and/or form tumors in appropriate animal hosts. The potential *in vivo* cooperation of tryptase with other mitogens in tumor growth will be investigated through *in vitro* mitogenesis studies with specific mitogens. Current work to delineate the mechanism of signal transduction for tryptase will continue, in order to better understand the biologic role of this mast cell-specific growth factor in the complex process of tobacco related carcinogenesis.

2023470943

Principal Investigator: Shibata

ABSTRACT

Direct Analysis of Lung Cancer Development and Progression

Title of Research Project (do not exceed 60 spaces)

Department of Pathology, USC School of Medicine

Department and Institution

ABSTRACT

The development of lung cancer caused by cigarette smoking is difficult to study since sequential lung biopsies are not easily acquired. Modern models of neoplasia stress the progressive but random accumulation of critical mutations which ultimately result in malignancy. These models predict that histologically distinct subsets of the tumor, carcinoma in situ, and dysplasia adjacent to the tumor should have different numbers of mutations. This prediction has not been rigorously tested since the numbers of residual pre-neoplastic cells from which the primary tumor arose are small and therefore technically difficult to isolate and examine.

A new method which allows the selection and specific analysis of DNA from a small number of cells on a stained fixed microscope section has been developed. The method involves the selective destruction and protection of DNA present on the microscope slide using ultraviolet radiation followed by PCR mediated analysis. Preliminary data suggests a resolution of approximately 20 cells with a theoretic ability to analyze one cell. This novel technique will be further developed and employed to detect mutations and loss of heterozygosity at the K-ras and p53 genes in dysplastic and neoplastic lung tissues. The method should allow the mapping of mutations to specific histologic regions in two dimensions and therefore directly document the legacy of tumor progression. It is estimated that at least 10-20 mutations are necessary for the development of lung cancer. The results obtained in this study should allow the investigation of additional loci as they arise in the future.

Principal Investigator: Coruh, Nursen

ABSTRACT

Protein Kinases: Unique Probes for the ATP Binding Site

Title of Research Project (do not exceed 60 spaces)

Department of Chemistry - University of California, San Diego

Department and Institution

Protein kinases are known to play a major regulatory role in all eukaryotic cells. They are critical for regulating numerous metabolic pathways, they are directly involved in many signal transduction pathways and they are key mediators of many cell cycle events. Furthermore many oncogenes code for protein kinases, and their transforming potential requires kinase activity. Over 200 protein kinases are now known. They differ in size, substrate specificity, subcellular localization, regulatory mechanism and subunit structure. Nevertheless, in spite of this diversity, all protein kinases share a conserved catalytic core, that indicates a common origin for all these important enzymes.

cAMP-dependent protein kinase (cAPK), is one of the simplest protein kinases, and the recently solved crystal structure of this kinase immediately establishes it as an ideal model for understanding many features that are common to all members of the protein kinase family. Of particular interest is the unique nucleotide binding motif that is found in the protein kinases. A dominant feature of this motif is a flexible glycine-rich loop that lies between 2 β-strands and binds to the β-phosphate of MgATP. This is a highly sensitive region. Mutations in this segment are known to induce oncogenic activation in protein kinases and in other cases can lead to kinase inactivation. The main objective of the proposed research is to develop a variety fluorescent probes to characterize structure-function relationships in the ATP binding domain of the catalytic (C) subunit of cAPK. One approach will involve introducing aromatic amino acids, namely tryptophans and tyrosines, into the segments of the protein that are in close proximity to the Mg(II)ATP binding site. These mutations will be utilized to follow the conformational changes in the enzyme upon Mg(II)ATP binding and upon peptide binding by monitoring the intrinsic fluorescence. A second approach will involve replacing the spectroscopically silent Mg(II) ions with highly luminescent lanthanide(III) (Ln(III)) ions, such as Tb(III), and Eu(III))in the ATP complex. Due to their intraconfigurational f→f transitions Tb(III) and Eu(III) ions are very sensitive probes for local conformational changes. The (mutagenic) aromatic groups mentioned above will also be utilized as donors in the energy transfer to the Tb(III) in the luminescence measurements from cAPK-bound Tb(III)ATP complex. The distance between donor and acceptor in this type of energy transfer is critical; therefore the energy transfer to the Tb(III) will only be from the nearest aromatic amino acid at the ATP binding site. A third approach for probing the ATP binding site in the C-subunit will be to introduce sulfhydryl selective fluorescent labels into strategically engineered mutant cysteine residues. Cysteines have been introduced already at 3 positions, F239, K192, and K81. In order to have a more global mapping of the protein cysteines will be introduced at the other positions in the protein during the proposed study. Energy transfer studies will be carried on between donor and acceptor pairs of appropriate fluorescent labels placed on catalytic and regulatory subunits of cAPK, and also on the protein kinase inhibitor (PKI).

Principal Investigator: Forman, Barry M.

ABSTRACT

RETINOID X RECEPTORS IN ONCOGENESIS

Title of Research Project (do not exceed 60 spaces)

THE SALK INSTITUTE / GENE EXPRESSION LABORATORY

Department and Institution

Cigarette smoking has been implicated in the development of cancers of the lung, oral cavity, esophagus as well as myelogenous leukemias. These tumors arise less frequently in individuals with increased serum carotene. The mechanism underlying this protective effect is unclear, but may be related to the anti-tumor effects of retinoids, a family of carotene metabolites that modulate development and oncogenesis. In humans, acute promyelocytic leukemia arises by expansion of a leukemic clone expressing a mutant retinoic acid receptor. The mutant receptor blocks differentiation at the promyelocyte stage. Retinoic acid allows differentiation to proceed to non-malignant neutrophils and has been used successfully to treat affected individuals. Similarly, the retroviral oncoprotein v-erbA, causes erythroblastosis by blocking erythrocyte differentiation. The oncogenic activity of v-erbA is related to its ability to inhibit transcriptional activation by retinoic acid receptors. The aim of this project is to determine the molecular mechanisms underlying the oncogenic activity of defective retinoid receptors.

Two classes of nuclear retinoid receptors have been identified. The first respond to low levels of retinoic acid. The second class require higher levels of retinoic acid for activity and are thought to mediate the biological actions of undefined retinoids. Both classes are members of the steroid/thyroid superfamily of nuclear receptors. These receptors contain distinct domains for DNA binding and dimerization. Recent evidence suggests that hetero-dimerization among mutant retinoid receptors leads to their oncogenic activity. This project will investigate the molecular mechanisms underlying normal dimerization and how alterations in dimerization lead to malignancy in genetically manipulated animals.

Specifically, selected retinoid receptors will be expressed and purified from *E. coli*. Their selective transcriptional activity will be analyzed *in vitro* and in transfected cells. Based on these studies, receptor mutants with alterations in specific transcriptional functions will be designed. The *in vivo* phenotype of these mutants will be determined using transgenic mice and gene-targeting technology. This approach will link retinoid receptor defects with their oncogenic phenotype and will provide insights into the role of carotene metabolites in the prevention and treatment of tobacco related malignancies.

Principal Investigator: Haselbeck, Robert J.

ABSTRACT

MDM9, a gene involved in yeast mitochondrial inheritance

Title of Research Project (do not exceed 60 spaces)

Department of Biology, University of California at San Diego

Department and Institution

The use of snuff or smokeless tobacco has been shown to correlate with the development of snuff dipper's leukoplakia, a pre-cancerous lesion characterized by abnormal expression and accumulation of keratin and keratin-based structures in epithelial cells of the oral mucosa. In the yeast *Saccharomyces cerevisiae*, temperature sensitive mutants in mitochondrial distribution and morphology (*mdm*) have been shown to rely in part on a cytoskeletal structure similar to keratin-based intermediate filaments found in epithelial cells. The protein product of one of these genes, *MDM9*, has been suggested to functionally interact with this structure. The following experiments are designed to determine the structure, expression and cellular function of the protein encoded by the *MDM9* gene by molecular genetic and biochemical means. The *MDM9* gene will be isolated by complementation of the mutant phenotype with a yeast genomic library. Translation of the *MDM9* coding region to determine the primary structure of the MDM9 protein will allow structural and functional comparisons with known protein sequences and functional motifs accessible from protein sequence data banks. Disruption of the *MDM9* chromosomal gene by homologous recombination will allow determination of the necessity of the MDM9 protein for normal cellular growth. An MDM9-specific polyclonal antiserum will be raised in rabbits. The cellular location of the MDM9 protein will be determined by indirect immunofluorescence analysis of whole cells and by Western blot analysis of subcellular fractions with the use of the anti-MDM9 antiserum. Cellular location of MDM9 as determined by these techniques will be correlated with that of MDM1 to help gauge the putative functional relationship between these two proteins. Further analysis of the interaction of the MDM1 and MDM9 proteins will be done by co-immunoprecipitation experiments using either anti-MDM1 or anti-MDM9 antisera. MDM9 expression in response to various growth stages and conditions will be determined by Western blot and Northern blot analyses. An *in vivo* assay of MDM9 cellular function will be developed based on the apparent reversibility of the *mdm9-2* mutant phenotype. These mutant cells will be observed by light microscopy for the effect of various inhibitors of cellular function on the renewed movement of mitochondria into buds after a heat challenge. These experiments will allow further elucidation of MDM9's role in mitochondrial inheritance as well as its putative interaction with structures established to comprise intermediate-filament-like structures in yeast.

Principal Investigator: Anning Lin

ABSTRACT

Isolation of an Oncogene-activated cJun Kinase Linking Ras to AP-1.

Title of Research Project (do not exceed 60 spaces)

Department of Pharmacology, Uiversity of California, San Diego

Department and institution

The pathogenesis of human lung cancer is a complex and multistep process, that is most likely initiated by genetic aberrations caused by carcinogens. The major etiologic agent for lung cancer is tobacco smoking, which contains a mixture of thousands of carcinogens. Activation of proto-oncogenes plays an important role in the development of the lung cancer. The most common activated proto-oncogenes are members of the ras family. An important mediator of ras action is another proto-oncogene c-jun, which encodes a component of the AP-1 transcriptional activator, is also activated in lung cancer. AP-1 is a complex composed of the Jun and Fos proto-oncogene products, forming both homo- and heterodimers. The activity of AP-1 is not only stimulated by tumor promotors and growth factors, but also augmented by transforming oncogenes. In the presence of activated Ha-Ras oncogene, cJun transforms immmortalized rat fibroblasts or primary rat embryo fibroblasts. The augmentation by Ha-Ras of cJun is due to stimulation of an unknown cellular Ser/Thr protein kinase which leads to inceased phosphorylation of cJun on two sites within its N-terminal activation domain. In this proposal, I would like to investigate the biochemical mechanisms underlying the oncogene cooperation between Ha-Ras and cJun. First, I will examine the phosphorylation of cJun in lung cancer cells in which *ras* proto-oncogenes are activated. Then, I will isolate the cellular cJun kinase responsible for phosphorylation of cJun on its N-terminal sites using conventional chromatography, such as ion exchange and gel filtration, and HPLC in combination with an in vitro phosphorylation assay and two-dimensional phosphopeptide mapping. Partial amino acid sequence of the cJun kinase will be determined and used to generate oligonucleotide to isolate the cDNA encoding the kinase. The cloned cJun kinase will be expressed in bacteria to obtain recombinant kinase. Polyclonal and monoclonal antibodies that specifically react with the kinase will be made and used to examine the cJun kinase activity during the development of lung cancer. This may provide a marker for the early Development of lung cancer.

2023470948

Principal Investigator: Churl K. Min

ABSTRACT

lecular mechanisms of brain nicotinic receptor inactivation

Title of Research Project (do not exceed 60 spaces)

Division of Biology, California Institute of Technology

Department and Institution

Nicotine, the drug in tobacco that causes addiction, is a powerful pharmacological agent that acts in a variety of ways at different sites in the body. Nicotine, which enters the brain, interacts with nicotinic acetylcholine receptors (nAChRs) in brain tissues, and initiate metabolic and electric activity. A number of studies have found that the chronic administration of nicotine causes an increase in the density of nicotinic receptors in the brain, accompanied by the development of tolerance, one of the many symptoms of nicotine addiction. One of the signals for up-regulation could be attributed to receptor desensitization, an agonist-induced conformational change due to prolonged exposure to agonist, which is largely responsible for the receptor inactivation. The synthesis of additional brain nAChRs may be a cellular compensation response to nicotine-induced receptor inactivation.

Using a heterologous expression system in Xenopus oocytes combined with electrophysiological measurement, structural changes will be investigated based on a "flap arrangement" model (Armstrong and Bezanilla, 1977) as a mechanism underlying the inactivation of brain nAChRs. The rationale behind this approach is that extracellular application of a synthetic peptide corresponding to the flap behaves like an open channel blocker (Demo and Yellen, 1991) of the brain nAChR. The receptor region for the flap will be located using the affinity labeling technique. Furthermore, this study will extend involvement of receptor phosphorylation in the inactivation of brain nAChRs, an effect that is firmly established in muscle-type nAChRs. In situ phosphorylation will be induced by activating the co-expressed seven transmembrane receptor. The phosphorylation of brain nAChRs is expected to increase the affinity for the flap, leading to increase in both the rate and extent of inactivation. To complement the in situ phosphorylation study, we will seek a method for moderate-level expression of brain nAChRs (~10 fmol/mg protein) using vaccinia virus. This method will provide enough quantities of brain receptors for in vitro phosphorylation and reconstitution studies.

Principal Investigator: Minden, Audrey, G.

ABSTRACT

Regulation of the NGFI-A Protein by Phosphorylation

Title of Research Project (do not exceed 60 spaces)

Department of Pharmacology. University of California - San Diego

Department and Institution

Normal cells must be able to respond to a variety of external stimuli which elicit different cellular responses. The rat pheochromocytoma PC12 cell line provides an excellent model for studying how external stimuli influence the cellular phenotype. These cells can be induced to differentiate into neuron like cells by a variety of factors including nerve growth factor (NGF), or to proliferate by epidermal growth factor (EGF). PC12 cells also undergo membrane depolarization in response to nicotine. NGF and EGF and nicotine all induce the rapid expression early response genes in these cells. It is important to understand how the products of these genes can mediate responses to such diverse signals, in order to understand the abnormalities that occur when cells fail to respond properly to external stimuli. In cancer, cells fail to respond properly to signals that induce proliferation or differentiation, resulting in uncontrolled proliferation. Nicotine addiction may also be caused by transcriptional changes that are mediated by the early response proteins induced by this drug. The mechanisms by which the products of early response genes can respond to such diverse stimuli are unknown. One possibility is that differential post-translational modifications of these proteins in response to different signals can modulate their functions. The experiments in this proposal are designed to determine whether differential post-translational modifications could explain how the product of one early response gene, NGFI-A (also known as Zif268, Krox24, Egr-1, and Tis8), can respond to diverse external stimuli. The NGFI-A protein, which is a sequence specific transcription factor, will be analyzed for differences in phosphorylation in response to EGF and NGF and nicotine in PC12 cells. Its ability to bind to its target DNA sequence and its activity as a transcriptional regulator after stimulation with these agents will be analyzed.

Principal Investigator: Sheridan, Philip L.

ABSTRACT

Biochemical and Functional Characterization of HIP-116

Title of Research Project (do not exceed 60 spaces)

Regulatory Biology Department, The Salk Institute for Biological Studies

Department and Institution

We have recently isolated a cDNA for a putative transcription factor, HIP116, which is highly related to *SNF2*, a transcription factor that is required for expression of numerous growth-inducible genes in *Saccharomyces cerevisae*. In addition, the HIP116 protein is related to several newly-isolated proto-oncogenes that are predicted to form an unusual reiterated "zinc finger" structure, and also possesses motifs related to the ATPase and DNA helicase domains of the SV40 large T (tumor) antigen. Taken together, these findings indicate that HIP116 is a conserved protein may play a very important role in the regulation of cell growth in mammalian cells. Moreover, these findings suggest that it may carry out this function through direct alteration of the promoter DNA (e.g., by unwinding) of the promoter DNA. Here I propose to develop in vitro assay systems in which to analyze the biochemical properties of the HIP116 protein, and to identify the domains of the protein necessary for transcriptional control both in vitro and in vivo.

Principal Investigator: SHUM, Lillian

ABSTRACT

Signal Transduction by TGF-α precursor during transformation

Title of Research Project (do not exceed 60 spaces)

Department of Growth and Development, University of California, San Francisco

Department and Institution

ABSTRACT

The use of tobacco has been associated with tumorigenesis of numerous types of epithelial carcinomas. In these tumor types, TGF-α is abundantly expressed, often concommitantly with its corresponding receptor. It has been suggested that signal transduction mediated by the receptor could eventually lead to malignant transformation.

The TGF-α precursor resembles a receptor-like molecule. It is a transmembrane protein with an EGF-like extracellular domain and highly conserved cysteine-rich cytoplasmic domain. Based on this structure and reinforced by some preliminary data, we hypothesize that the TGF-α precursor has signal transducing capabilities, such that interactions with its receptor result in a bidirectional cell cell communication, which may play a role in epithelial proliferation and transformation. Our research project will investigate the role of the cytoplasmic domain of the transmembrane TGF-α precursor. We will evaluate the question of where this signal is being generated by the TGF-α precursor in polarized epithelial cells, i.e whether the TGF-α precursor and its receptor are at the apical versus the basolateral surfaces of the epithelial cells. We shall also evaluate the nature of this signal through the transmembrane TGF-α by examining representative early signal transduction events such as inositol phosphate metabolism and calcium mobilization, and changes in transcriptional regulation. Finally, we shall use site-directed mutagenesis to define structural elements in the cytoplasmic domain of the TGF-α precursor which are important to this signaling. The results of this study should provide insight into the physiological significance of TGF-α precursor as a signaling molecule and its contribution to epithelial transformation.

2023470952

Principal Investigator: Rati Verma, Ph.D.

ABSTRACT

Cell cycle regulation of proliferation genes

Title of Research Project (do not exceed 60 spaces)

Divisions of Chemistry and Biology, California Institute of Technology

Department and Institution

Tobacco, either alone, or in combination with alcohol, accounts for about 35% of the cancer cases occurring in the United States today (Doll and Peco, 1981). Most cancers arise from deregulated growth of cells which is a consequence of either activation of proto-oncogenes or inactivation of normal suppressor genes serving to constrain cell growth. An understanding of the normal cell-division-cycle with its built in regulatory mechanisms to ensure fidelity of division is therefore crucial to understanding the origins of cancer.

One of the master regulators of the cell cycle, the *CDC28* protein kinase was originally identified in yeast. Since then, homologs of this kinase (cyclin-dependent kinases; CDKs) have been found to be conserved through phylogeny. Together with the cyclins, the *CDC28* gene product controls entry into the S phase (where DNA duplication takes place) as well as the mitotic (nuclear division) phases of the cell cycle. Between the *CDC28* commitment step in G1 and the initiation of DNA replication in S phase, there is an induction in the message levels of 13 key replication genes at the G1/S boundary of the cell cycle.

What confers this periodicity to the DNA replication genes? As recently pointed out by several workers, all 13 of these genes have at least one copy of the sequence 5' ACGCGT 3' in their promoters. That this sequence is indeed important.for cell cycle regulation has been conclusively demonstrated in this laboratory using the DNA polymerase I promoter. DNA polymerase I is a key enzyme of the replication fork involved in DNA synthesis initiation and elongation. The elucidation of the *cis*-acting element conferring periodicity to POL1 has set the stage for the identification and isolation of factor(s) that interact with it.

Using yeast nuclear extracts fractionated by conventional and oligonucleotide affinity chromatography, a specific cell cycle element binding protein, designated MCBF, has been purified to near homogeneity. Polyclonal antisera has been raised against MCBF. λgt11 expression library is currently being screened with the antibody to isolate the gene.

The mechanism by which MCBF confers periodicity to DNA replication genes is also currently under investigation. Using unfractionated yeast extracts prepared from synchronized cultures as well as from several cell division cycle mutants it has been shown that binding to the cell cycle element is periodic. A novel binding activity has been identified. Future experiments will be focused on screening a yeast expression library with a radiolabeled, cell cycle element DNA binding probe. Isolating the MCBF and MCBF-like genes by such an approach and creating mutants in each will help us elucidate downstream events such as initiation of DNA replication. Upstream events, such as whether cdc28 protein kinase (the evolutionarily conserved cell cycle master regulator) activates the *trans*-acting factors will also be studied. These series of experiments will further our understanding of the normal cascade of cell cycle events. This in turn is crucial for understanding how regulated checkpoints of the cell cycle are bypassed when the cell becomes oncogenic, proliferating uncontrollably.

Principal Investigator: YEO, HELEN C.H.

ABSTRACT

DNA Damage in Germ and Somatic Cells of Smokers

Title of Research Project (do not exceed 60 spaces)

Molecular and Cell Biology, University of California, Berkeley

Department and Institution

Tobacco smoke is known to contain high levels of oxidants. Some of these oxidants are known mutagens and are capable of damaging DNA, leading to cancer. This proposal attempts to develop a non-invasive assay to measure DNA adducts in the sperm of smokers as it would pose a risk factor for paternally induced cancers and birth defects. This finding would provide evidence that smokers are damaging their offspring. Damaged DNA is constantly repaired by DNA repair enzymes and the excised adducts are excreted in the urine where they can be assayed. Under extreme oxidative stress such as heavy smoking, we hypothesize that levels of DNA adducts in sperm and urine will be elevated.

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography with nitrogen phosphorus detection (GC-NPD) will be used as analytical tools for the detection and identification of total DNA adducts in sperm to assess the production of oxidative DNA damage *in vivo* associated with smoking. Using monoclonal antibody columns and GC-MS, the search for DNA adducts excreted in the urine of smokers will be evaluated. Furthermore, an assay using gas chromatography-mass spectrometry with selective ion monitoring (GC-MS-SIM) will be developed to measure 4-hydroxy-8-oxo-7,8-dihydroguanosine (oh^4oxo^8dG), an adduct induced by singlet oxygen, in sperm DNA and urine.

2023470954

DOOR

Principal Investigator: Hye-Joo Yoon, Ph.D.

ABSTRACT

The Mechanics and Regulation of Centromere Function

Title of Research Project (do not exceed 60 spaces)

Department of Biological Sciences, University of California, Santa Barbara

Department and Institution

The culmination of the eukaryotic cell cycle is often described as the molecular mechanical events of M phase, during which chromosome segregation and nuclear division occur. The process of chromosome segregation at mitosis and meiosis is crucial to the faithful execution of normal cell division and is therefore a key aspect of cell biology. Proper segregation of eukaryotic chromosomes depends upon a centromere/kinetochore. In *Saccharomyces cerevisiae*, all the DNA sequences needed in cis to specify full centromere function occur within a 125-bp DNA segment (*CEN* DNA) that is organized into three conserved DNA elements, CDEI, CDEII, and CDEIII. It seems likely that the protein(s) binding to the 25 bp CDEIII region of the *CEN* DNA plays a major role in centromere/kinetochore function, since single base changes in this sequence can completely inactivate the centromere. Recent research in Dr. Carbon's laboratory has led to the discovery of a novel centromere binding protein called CBF3. Native CBF3 is a 240 kDa protein complex composed of three subunits and binds specifically to the wild-type CDEIII sequence but not the inactive mutated sequence. Biochemical studies demonstrated that CBF3 carries considerable ATPase activity and must be phosphorylated for *CEN* DNA binding to occur. Furthermore, preliminary data show the presence of a microtubule-dependent motor activity in CBF3. These findings, if verified, suggest that the budding yeast centromere contains a molecular motor whose activity is regulated by phosphorylation.

In an attempt to understand the functional roles of CBF3 in the kinetochore, the genes encoding the three CBF3 proteins are now being isolated and mutated to look for a relevant phenotype. I propose to examine possible mechanisms of regulation of CBF3 function throughout the cell cycle. To do this, CBF3-specific antibodies will be used as a tool in following the protein abundance, phosphorylation state, and subcellular localization in a cell cycle-dependent manner. A microtubule-dependent motor activity of CBF3 will also be assayed by using pure proteins isolated from cells blocked at different stages of the cell cycle. In addition, genetic and biochemical approaches will be taken to identify other components of the kinetochore. Assuming some of these components interact with CBF3, this will be done by the use of protein affinity columns with CBF3 bound to a suitable column matrix or by searching for extragenic suppressors of *cbf3* mutants.

TOBACCO-RELATED DISEASE RESEARCH PROGRAM

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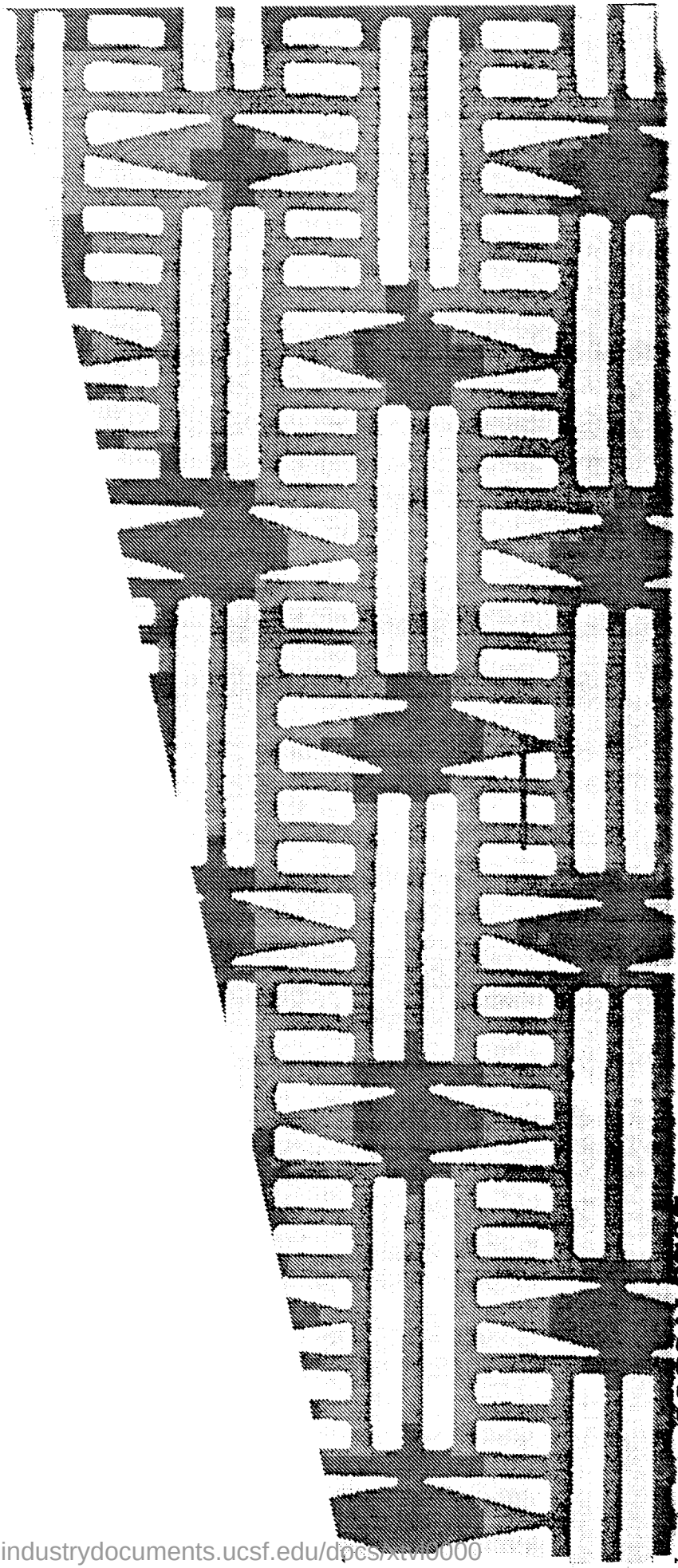
TOBACCO-RELATED DISEASE RESEARCH PROGRAM

July 1992

Grants Awarded In

Cardiovascular Disease, Pulmonary Disease, and Cardiovascular and Pulmonary Disease Career Development

University of California
Office of Health Affairs



TRDRP

Tobacco-Related Disease Research Program

July 1992

Grants Awarded In

Cardiovascular Disease, Pulmonary Disease, and Cardiovascular and Pulmonary Disease Career Development

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University of California

In November 1988, California voters approved Proposition 99, the Tobacco Tax and Health Protection Act, which instituted a 25¢-per-pack cigarette surtax. This initiative specified that five percent of the revenue be deposited into a Research Account, to be appropriated for research on tobacco-related disease. The Tobacco-Related Disease Research Program was established by passage of SB 1613 (Chapter 1330 of the Statutes of 1989) in October 1989: "The Legislature hereby requests the University of California to establish a comprehensive grant program to support research efforts related to the prevention, causes, and treatment of tobacco-related diseases" (Section 424.20 of the Health and Safety Code).

Within the Office of the President of the University of California, specific responsibility for administering this research program was assigned to the Vice President—Health Affairs, Cornelius L. Hopper, M.D., who established the Tobacco-Related Disease Research Program (TRDRP). TRDRP has responsibility for the management of all fiscal and programmatic aspects of the grant program, while the Research Administration Office in the Office of the President manages all contractual relationships with institutions other than University of California campuses.

Direction and oversight of TRDRP are provided by a Scientific Advisory Committee which comprises representatives of various institutions with interest in the program. A list of members and the institutions they represent appears at the end of this booklet.

Research funds are available to investigators at all non-profit research institutions in California. Grant applications are peer reviewed by panels of leading investigators drawn from research institutions throughout the country. The evaluation procedure is modeled on the one used by the National Institutes of Health. In 1992, 132 reviewers evaluated applications in the following nine study sections:

Behavioral and Public Health
Carcinogenesis
Cardiovascular Disease
Epidemiology
General Biomedical Science
Pulmonary Disease
Tumor Immunology and Therapy
Cancer and Biomedical Science Career Development
Cardiovascular and Pulmonary Disease Career Development

Study sections evaluated the scientific merit of proposals and rated their responsiveness to TRDRP's current research priorities. The Scientific Advisory Committee reviewed these evaluations and recommended funding levels to the UC Vice President—Health Affairs.

In 1992 TRDRP awarded approximately $24 million to investigators at 25 institutions. The 87 grants awarded constituted more than 21 percent of applications received. From July 1990 to date, TRDRP has awarded 376 grants, for a total of approximately $118 million, to investigators at 40 California research institutions. TRDRP issues an annual report to the California Legislature which includes reports of scientific progress on active grants.